LIGHT-EMITTING DIODE LIGHTING PARAMETRIC STUDY OF INTERNALLY ILLUMINATED PHOTOBIOREACTOR FOR GOOD GROWTH OF NANNOCHLOROPSIS SPECIES CULTIVATION

MOHAMAD TAISIR BIN ABDULLAH

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DEDICATION

This thesis is dedicated to my mother, father, wife and kids. Thanks for your love, support and encouragement.

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ABSTRACT

Microalgae such as *Nannochloropsis* sp. are single-cell organism, well known as a photosynthetic microorganism and have been identified as one of the most potential renewable feedstock for the third generation biofuels. Light quality and quantity are essential for a good growth of microalgae cultivation. In this research, lighting parametric study using light-emitting diode (LED) was conducted to analyse the effect of different light spectrum onto the growth of *Nannochloropsis* sp. cultivation. In addition, the effect of light intensity with different optical path length on different working volume culture was also evaluated. In order to validate a good growth of cultivation, culture's growth curve was analysed and maximum cell density was recorded. The study was performed in three stages of experimental photobioreactor (PBR) setup which is lab-scale, mock-up and scale-up PBR. In the lab-scale experiment (0.5 L working volume), LED with red spectrum (wavelength 660 nm) and blue spectrum (wavelength 457 nm) were compared to the white fluorescent light with same incident light intensity (100 $\mu mol\, m^{-2}s^{-1}$) at short optical path length (20 to 55 mm). It was found that LED with combination of red and blue spectrum generated higher maximum cell density by 19% compared to the white fluorescent light, which recorded at 11.2 $\times 10^6$ $cells\, mL^{-1}$. In the mock-up PBR experiment (20 L working volume), light intensity of red and blue LED module with narrow beam angle (55 °) was evaluated within 15 to 120 mm of optical path length by using variation of current supply (200 to 500 mA). As the result, the maximum cell density recorded is 7.1 $\times 10^6$ $cells\, mL^{-1}$ at 355 $\mu mol\, m^{-2}s^{-1}$ of light intensity. Additionally, the relationship between light intensity and culture cell density was also established at this stage. Next, the cultivation was performed continuously in the scale-up PBR with bigger working volume (30, 65 and 100 L) at 100 mm optical path length. It was found that light saturated condition happened at cell density around 7.5 $\times 10^6$ to 8.0 $\times 10^6$ $cells\, mL^{-1}$ when the light intensity is at 350 $\mu mol\, m^{-2}s^{-1}$. The maximum cell density can be increased further to 9.3 $\times 10^6$ $cells\, mL^{-1}$ by applying higher light intensity (450 $\mu mol\, m^{-2}s^{-1}$). As a conclusion, LED with red spectrum promoted the growth in the exponential growth phase, while blue spectrum had a significant role during the linear growth phase especially in the higher cell density culture. Application of high intensity LED light with narrow beam angle was feasible to be used in internally illuminated PBR with longer optical path length. On top of these findings, a vertically stackable LED luminaire design concept was proposed to provide flexibility and to increase efficiency for mass cultivation operation using internally illuminated PBR.

ABSTRAK

Mikroalga seperti *Nannochloropsis* sp. adalah organisma sel tunggal, terkenal sebagai mikroorganisma fotosintetik dan telah dikenal pasti sebagai salah satu bahan mentah boleh diperbaharui yang paling berpotensi untuk bahan api bio generasi ketiga. Kualiti dan kuantiti cahaya adalah penting untuk pertumbuhan yang baik bagi penternakan mikroalga. Dalam penyelidikan ini, kajian parametrik pencahayaan menggunakan diod pemancar cahaya (LED) telah dijalankan untuk menganalisa kesan spektrum cahaya yang berbeza terhadap pertumbuhan penternakan *Nannochloropsis* sp. Di samping itu, kesan keamatan cahaya dengan panjang laluan optik yang berbeza pada isipadu kerja kultur yang berbeza juga dinilai. Untuk mengesahkan pertumbuhan yang baik dalam penternakan, lengkung pertumbuhan kultur dianalisa dan ketumpatan sel maksimum direkodkan. Kajian telah dijalankan dalam tiga peringkat persediaan ujikaji fotobioreaktor (PBR) iaitu PBR skala makmal, skala kecil dan skala besar. Dalam ujikaji skala makmal (isipadu kerja 0.5 L), LED dengan spektrum merah (panjang gelombang 660 nm) dan spektrum biru (panjang gelombang 457 nm) dibandingkan dengan lampu kalimantang putih dengan keamatan cahaya tuju yang sama (100 $\mu mol\, m^{-2}s^{-1}$) pada panjang laluan optik yang pendek (20 hingga 55 mm). Didapati bahawa LED dengan gabungan spektrum merah dan biru telah menghasilkan ketumpatan sel maksimum yang lebih tinggi sebanyak 19% berbanding lampu kalimantang putih, yang telah dicatatkan pada 11.2×10^6 $cells\, mL^{-1}$. Dalam ujikaji PBR skala kecil (isipadu kerja 20 L), keamatan cahaya modul LED merah dan biru dengan sudut pancaran sempit (55 °) telah dinilai di antara 15 hingga 120 mm panjang laluan optik dengan menggunakan variasi bekalan arus (200 ke 500 mA). Hasilnya, ketumpatan sel maksimum yang direkodkan adalah 7.1×10^6 $cells\, mL^{-1}$ pada keamatan cahaya 355 $\mu mol\, m^{-2}s^{-1}$. Di samping itu, hubungan antara keamatan cahaya dan ketumpatan sel kultur juga telah diperolehi pada peringkat ini. Seterusnya, penternakan dijalankan secara berterusan dalam PBR skala besar dengan isipadu kerja yang lebih besar (30, 65 dan 100 L) pada panjang laluan optik 100 mm. Didapati bahawa keadaan tepu cahaya berlaku pada ketumpatan sel sekitar 7.5×10^6 ke 8.0×10^6 $cells\, mL^{-1}$ apabila keamatan cahaya adalah pada 350 $\mu mol\, m^{-2}s^{-1}$. Ketumpatan sel maksimum boleh ditingkatkan lagi kepada 9.3×10^6 $cells\, mL^{-1}$ dengan menggunakan keamatan cahaya yang lebih tinggi (450 $\mu mol\, m^{-2}s^{-1}$). Sebagai kesimpulan, LED dengan spektrum merah menggalakkan pertumbuhan dalam fasa pertumbuhan eksponen, manakala spektrum biru mempunyai peranan penting semasa fasa pertumbuhan linear terutamanya dalam kultur berketumpatan sel lebih tinggi. Penggunaan lampu LED berkeamatan tinggi dengan sudut pancaran sempit sesuai digunakan dalam PBR bercahaya dalaman dengan panjang laluan optik yang lebih panjang. Di samping penemuan ini, konsep reka bentuk lampu LED boleh tindanan menegak telah dicadangkan untuk memberikan fleksibiliti dan meningkatkan kecekapan untuk operasi penternakan besar-besaran menggunakan PBR bercahaya dalaman.

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LIST OF ABBREVIATIONS

AFDW	-	Ash Free Dry Weight
ALP	-	Airlift Photobioreactor
CCT	-	Correlated Color Temperature
EPA	-	Eicosapentaenoic Acid
FAME	-	Fatty Acid Methyl Esther
HLTP	-	Horizontal Loop Tubular Photobioreactor
LED	-	Light-emitting Diode
MFPP	-	Modular Flat Panel Photobioreactor
PAR	-	Photosynthetically Active Radiation
PBR	-	Photo Bioreactor
PC	-	Polycarbonate
PCB	-	Printed Circuit Board
PE	-	Polyethylene
PFD	-	Photon Flux Density
PMMA	-	Polymethyl Methacrylate
PP	-	Polypropylene
PPFD	-	Photosynthetic Photon Flux Density
PVC	-	Polyvinyl Chloride
SMD	-	Surface Mount Devices
UV	-	Ultra Violet

LIST OF SYMBOLS

μ	-	Growth rate
N	-	Cell densities
t	-	time
k	-	Division rate
V_c	-	Working volume of culture
V_t	-	Volume of tank
V_i	-	Volume of inner column
d_t	-	Diameter of tank
d_i	-	Diameter if inner column
h_c	-	Height of culture
IS	-	Illumination surface
BS	-	End of illumination surface (opposite surface to the IS)
h	-	hours
vvm	-	Vessel volumes per minute
v/v	-	Volume per volume

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CHAPTER 1

INTRODUCTION

1.1 Background

With the increasing demand of energy and high rate of CO_2 emission globally, renewable energy is expected to be not only as an alternative, but sustainable and clean energy source [1]. In transportation sector, renewable energy such as biofuels has emerged as solution to the increasing price and limited source of fossil fuel. They are theoretically and practically sustainable, renewable, environmentally cleaner, biodegradable and have significant economic potential compared to the fossil fuel [2]. First generation biofuels are mainly limited to ethanol which is derived from starch, such as from feedstock of corn and potato (food source). Second generation biofuels are produced from non-food residues such as agricultural biomass which contains cellulose or lignin. While third generation biofuels are mainly produced from specially engineered crops, algae and microbial [3]. Commercially available biofuels today can be divided mainly into bioethanol and biodiesel. Currently United States, Brazil, Argentina and European Union are among the largest producers of biofuels.

Biodiesel can be used in unmodified diesel engines, and has advantages over fossil diesel fuel in that it produces less SO_x and particulate emissions when combusted. Compared to the conventional biodiesel which is mainly produced from vegetable or crops oil, algae-based biodiesel is categorised under advanced biofuel technology as the conversion technology is still undergoing extensive research and development process [4].

Microalgae offer advantages over crops such as simple growth requirement, short growth cycles, high lipid content and ease of being modified by biotechnological means. They are mainly divided into prokaryotic and eukaryotic.

Prokaryotic contains no chloroplas, no nucleus and have different gene structure compared to eukaryotic. They are largely known as cyanobacteria (blue-green bacteria) which are able to assimilate atmospheric nitrogen, but do not produce significant quantities of storage lipid. Meanwhile, eukaryotic division consists of several groups which differ in pigment composition, biochemical constituents, ultrastructure and life cycle. Among the groups are Rhodophyta (red algae), Chlorophyta (green algae), Dinophyta (red-brown algae), Chrysophyta (golden algae) and Eustigmatophyta (eyespot algae). Microalgae can be found in both freshwater and seawater (saline) habitats [5].

There are at least two ways of microalgae growth; heterotrophic and phototrophic. In heterotrophic cultivation, microalgae utilize organic carbon sources for chemosynthesis process, without the needs of light energy. While in phototrophic growth, microalgae will transform carbon dioxide and light energy through photosynthesis into various forms of chemical energies [6]. Phototrophic microalgae cultivation can be divided to two kinds; outdoor cultivation system and indoor cultivation system. In outdoor cultivation, the system utilize the sunlight as the primary light source such as the popular open pond system and recently emerging the photobioreactor (PBR) system. On the other hand, indoor cultivation systems are being managed in a relatively well controlled environment or enclosed PBR, and the light source depends on artificial lights such as fluorescent light, high-pressure sodium, metal halide and light-emitting diode (LED) [7].

In terms of light aspect, there are several issues which are related to the cultivation of microalgae. Among them are gradiation of light, inconsistent lighting period, depth of light penetration, cell mutual shading and photoinhibition. These issues are generally discovered at the outdoor cultivation system, which is directly related to the nature of sunlight as the primary light source. Whereas for indoor cultivation system which utilizes the artificial light as the light source, issues such as wavelength or light spectrum, light intensity, light uniformity, form factor of luminaire, economics and energy consumption are been highlighted [4].

1.2 Problem Statement

An efficient, robust yet practical with economical cost, easiness of usage and maintenance are among important criteria required for a photobioreactor (PBR) to cultivate microalgae in commercial scale. Conventional open ponds (outdoor system) method have apparently reached their upper limit to be improved further, mainly due to the low density culture, difficulty on contamination control and highly fluctuated by environmental variables [8]. Enclosed PBR running at outdoor (using sunlight energy) has emerged as solution to the contamination issue, however at certain level it is still influenced by environmental factors especially on light and temperature aspect [9]. Unfortunately, current solutions provided to improve these issues imposed another complexity in design and engineering, and in few cases failed to perform in commercial scale [10].

In this respect, enclosed PBR running at controlled environment (indoor) would provide a potential solution. Due to restriction of sunlight availability in indoor environment, the usage of artificial light has become crucial and need for the details of study at the basic principle and application level.

1) It is quite difficult to achieve similar characteristics of sunlight by using the artificial light, in term of energy (intensity), uniformity, spectrum range and so on. One of the steps in order to replace the sunlight is to find an artificial light which can accommodate the basic requirements of microalgae to grow. Photosynthetic response of chlorophyll pigments in plants and microalgae have been studied by researchers, and based on this various studies have attempted to proposed a suitable light. For an example, some studies proposed the usage of fluorescent light, but there is lack of analysis to determine the suitable correlated color temperature (CCT). Recently, with the availability of LEDs in various color (spectrum), numerous studies have been done on microalgae cultivation. However, from the overall view it was found that the results are varied for each kind of microalgae species. As such an analysis and recommendation is

needed in order to select a suitable type of artificial light to replace the function of sunlight for an indoor microalgae cultivation.

2) In actual situation of microalgae cultivation, in term of lighting aspect the microalgae growth condition does not rely on the subject of spectrum solely. Rather, it is a combination of other lighting factors as well such as intensity, penetration depth, coverage area and so on. These factors become more crucial if the volume of microalgae cultivation is increased (longer optical path length) or at higher density of microalgae. Due to this, there is necessity to understand the inter-relation between the light characteristic, volume of the culture and density of microalgae, especially for an indoor system which concern on the energy consumption.

3) Each type of PBR has their advantages or disadvantages in terms of microalgae cultivation efficiency and has been widely studied before, especially for outdoor environment. However there is lack of study in relation to the suitability of lighting luminaire for PBR at indoor environment. In general, the usage of readily available lighting luminaire onto PBR may not be applied easily due to the difference of application which it has been designed originally. There should be a design consideration in order to maximize the transfer of light energy to the microalgae, easiness of usage for daily operation and routine maintenance. Based on the understanding of the light characteristic, microalgae growth condition and cultivation process in actual PBR, there is a requirement to propose and develop a suitable lighting luminaire for such application.

4) In general, there is still lack of commercialization in the microalgae cultivation industry in Malaysia, especially for the biofuel application which required expertise in the upstream and downstream process. In terms of mass cultivation at outdoor environment, fluctuation of weather (high temperature and raining) is one of the main

consideration and risk factor. While in the indoor environment, there is still lack of study on the feasibility for the medium to high volume scale of cultivation which required by commercial level.

1.3 Research Questions & Objectives

Enclosed PBR has been actively developed since few decades as alternative to conventional open ponds system. Flat plate, tubular and column type PBR are considered as the main configuration of most PBR available today [11]. All these PBRs are developed to improve open ponds system, with the light source is sunlight rays – outdoor cultivation in mind. The main factor in determining suitable PBR for this case is to select the high surface to volume ratio, in order to increase the capacity of capturing solar rays (external illumination) [12]. On the other hand, enclosed PBR which is being used indoor depends on artificial light which need a different approach since the light and energy source are limited. Thus, below are the objectives of this research:

1) To study the effect of different light spectrum (wavelength) provided by LED light on the growth and lipid content of *Nannochloropsis* sp. cultivation; compared with the standard white fluorescent light. The study was conducted in a lab scale condition (working volume 0.5 *L*).

2) To investigate the LED light characteristic in deeper volume (longer optical path length) and the relationship between light intensity to the volume of culture and microalgae density. The study was conducted in a mock-up PBR (working volume 20 *L*) using internally illumination method with selected light spectrum and beam angle.

3) To verify the good growth of *Nannochloropsis* sp. cultivation by using LED light in an internally illuminated column PBR from medium to high volume (working volume 30 to 100 *L*).

1.4 Research Scope

The research was conducted within the following scopes:

1) *Nannochloropsis* sp. is the microalgae strain being used for this research due to the high photosynthetic efficiency and lipid productivity characteristic. The *Nannochloropsis* sp. is cultured in natural seawater enriched with Walne's medium. The cultivation process is conducted in an indoor environment (controlled room) with room temperature within 23 to 25 $°C$, while pH was monitored and controlled within 7 to 8. In each experiment, the sub-culture was being prepared up to 10% from the total working volume.

2) LED is being used for this research due to the high energy efficiency characteristic and multi options in terms of light wavelength and beam angle compared to other type of artificial light source. The light spectrum which being selected are blue (457 nm) and red (660 nm), while the beam angle is in the range of 55 to 130°.

3) Column type PBR is being chosen as a basic form factor of PBR due to its suitability for the application of internal illumination method (indoor cultivation type). The working volume used for the cultivation in PBR is in the range of 20 to 100 L, while the optical path length is within 100 to 120 mm.

1.5 Research Significance

This research is expected to contribute in solving some of the industrial issues in attempting for commercialization and mass cultivation of microalgae indoor, particularly on the aspect of lighting parameters selection (spectrum, intensity, etc) and luminaire development (optical beam, optical path length, etc), as well as configuration of PBR (form factor, size, volume, illumination method, etc). In future, it is expected to lead a way to commercialize the microalgae cultivation industry in Malaysia, not only for the application of biodiesel but also for other potential segments such as pharmaceuticals, nutrition, pigments, aquaculture or CO_2 sequestration [13].

1.6 Thesis Outline

This thesis contains six chapters including this chapter (Chapter 1). In Chapter 2, there are mainly three parts of the literature review. The first part presents the general overview of microalgae and cultivation process in general. The second part presents the overview of open pond and various PBR which have been developed to cultivate microalgae in outdoor and indoor. Meanwhile, in the third part it reviews the light aspect which is related to the cultivation of microalgae and summary of artificial light usage in the past studies.

Chapter 3 explains the methodology used to conduct the research. In this chapter, the research flowchart is explained at the beginning of the chapter followed by the description of each experiment's method. In general, the experiments are being divided to three different stages, which is lab scale experiment, followed by mock-up PBR experiment and finally scale-up PBR experiment. The parameter in each experiment is presented in this chapter, while the considerations which were taken for each stage are discussed in the next chapter.

Chapter 4 presents the result and analysis of each experiment, while at the same time the considerations for the following experiment's parameters are being discussed. Comparison of the result between our study and others are being presented accordingly.

Chapter 5 concludes the research findings. Recommendations and suggestions to advance the research work are stated in this chapter.

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LIST OF PUBLICATIONS

1. **Mohamad Taisir**, Chee Loong Teo, Ani Idris, and Affendi M. Yusuf. (2016). Cultivation of Nannochloropsis Sp. Using Narrow Beam Angle Light Emitting Diode in an Internally Illuminated Photobioreactor. Bioresources and Bioprocessing 3(1).
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3. Teo, Chee Loong, Madiha Atta, Attaullah Bukhari, **Mohamad Taisir**, Afendi M. Yusuf, and Ani Idris. (2014). Enhancing Growth and Lipid Production of Marine Microalgae for Biodiesel Production via the Use of Different LED Wavelengths. Bioresource Technology 162:38–44.