# BIOSYNTHESIS OF CARBOHYDRATE AND LIPID IN *Chlorella vulgaris* UPSI-JRM01 FOR BIOFUEL FEEDSTOCK PRODUCTION

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

2020

# BIOSYNTHESIS OF CARBOHYDRATE AND LIPID IN *Chlorella vulgaris* UPSI-JRM01 FOR BIOFUEL FEEDSTOCK PRODUCTION

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THESIS SUBMITTED IN FULLFILLMENT OF THE REQUIREMENT FOR THE
DOCTOR OF PHILOSOPHY
(RESEARCH MODE)

FACULTY OF SCIENCE AND MATHEMATICS
UNIVERSITI PENDIDIKAN SULTAN IDRIS

2020

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# ACKNOWLEDGEMENT

All praises to Allah for his blessing throughout my life and upon completion of this thesis. My greatest appreciation goes to my supervisor, Assoc. Prof. Dr. Norjan Yusof for her continuous supervision, concern, support and guidance from starting until completion of my study. I would like to express my special thanks to Assoc. Prof. Toshinari Maeda for supervision and guidance while I was doing research in Kyushu Institute of Technology, Japan. I would also like to thank my lab mates at Bioprocess and Maeda Laboratories and all the staffs and lecturers at the Biology Department for their assistance and made my experience at UPSI a wonderful journey. In addition, a special thanks to my beloved family for their encouragement and supports during my study. Last but not least, I would like to thank The Ministry of Education Malaysia (MOE) for financing this study through the Fundamental Research Grant Scheme (Code: 2015-01611-102-02). I am also grateful to Makmal Pencirian Struktur Molekul (MPSM) at the Centre for Research and Instrumentation Management (CRIM), Universiti Kebangsaan Malaysia (UKM) for their technical assistance.

# ABSTRACT

This study aims to investigate the effect of photo-autotrophic cultural conditions (light intensity, temperature, pH, $CO_2$ and $NO_3^-$) of *C. vulgaris* UPSI-JRM01 and to optimise the lipid, carbohydrate and biomass productivities for high biofuel feedstock production. In addition, carbon partitioning mechanism under nitrogen stress was also elucidated. The method of single-factor experiment was used to determine the effect of each cultural condition. The optimisation process was performed using statistical method of Plackett-Burman Design and Central Composite Design. The whole transcriptome analysis of gene expression under nitrogen stress was also performed using RNA sequencing. The results indicated that the lipid and carbohydrate yields were increased 3.19-fold and 1.39-fold under nitrogen stress and 5% $CO_2$, respectively. The highest biomass productivity was achieved at 10,500 lux, 28 $^{o}$C, pH 8, 5% $CO_2$ and 500 mg/L $NO_3^-$. Meanwhile, the highest lipid yield was achieved at 23,500 lux, 40 $^{o}$C, pH 8, 0.03% $CO_2$ and without $NO_3^-$ addition. The biomass, lipid and carbohydrate productivities were optimised to 404.24 mg/L/day, 65.30 mg/L/day and 165.43 mg/L/day, respectively. The major fatty acid methyl ester components were C16:0 (33.54%) and C18:2 (30.29%), thereby producing biodiesel complied with ASTM D6751 standard. Moreover, the results of gene expression study revealed the two-stage response to nitrogen stress; i) carbohydrate accumulation, plastid protein degradation, and amino acid biosynthesis, and ii) lipid accumulation, carbohydrate degradation, and DNA damage. In conclusion, the production of biofuel feedstock from microalgae biomass was feasible. The nitrogen stress triggered high carbohydrate accumulation before the carbon partitioned into triacylglycerol (TAG) using two different pathways; chloroplastic TAG synthesis and glycerolipid metabolism. The implication of this study can be associated with the potential development of biofuel feedstock from microalgae for future application and providing insight of carbon partitioning mechanism in *C. vulgaris* UPSI-JRM01 under nitrogen stress.

# BIOSINTESIS KARBOHIDRAT DAN LIPID OLEH *Chlorella vulgaris* UPSI-JRM01 UNTUK PENGHASILAN STOK SUAPAN BAHAN BAKAR BIO

## ABSTRAK

Kajian ini bertujuan mengkaji kesan keadaan kultur fotoautotrofik (intensiti cahaya, suhu, pH, $CO_2$ dan $NO_3^-$) *C. vulgaris* UPSI-JRM01 dan mengoptimumkan produktiviti lipid, karbohidrat dan biojisim untuk penghasilan stok suapan bahan bakar bio yang tinggi. Di samping itu, mekanisme pengagihan karbon dalam keadaan stres nitrogen juga dihuraikan. Kaedah eksperimen faktor tunggal digunakan untuk mengenal pasti kesan setiap keadaan kultur. Proses pengoptimuman dilakukan dengan menggunakan kaedah statistik Reka Bentuk Plackett-Burman dan Reka Bentuk Komposit Pusat. Pengekspresan gen melalui analisis transkriptom keseluruhan dalam keadaan stres nitrogen juga turut dilakukan menggunakan penjujukan RNA. Dapatan kajian menunjukkan bahawa hasil lipid dan karbohidrat meningkat 3.19 kali ganda dan 1.39 kali ganda dalam keadaan stres nitrogen dan 5% $CO_2$, masing-masing. Produktiviti biojisim tertinggi telah dicapai pada 10,500 lux, 28 $^oC$, pH 8, 5% $CO_2$ dan 500 mg/L $NO_3^-$. Sementara itu, hasil lipid tertinggi telah dicapai pada 23,500 lux, 40 $^oC$, pH 8, 0.03% $CO_2$ dan tanpa penambahan $NO_3^-$. Produktiviti biojisim, lipid dan karbohidrat pada keadaan optimum adalah 404.24 mg/L/hari, 65.3 mg/L/hari dan 165.43 mg/L/hari, masing-masing. Komponen utama asid lemak metil ester adalah C16:0 (33.54%) dan C18:2 (30.92%), seterusnya menghasilkan biodiesel yang mematuhi standard ASTM D6751. Selain itu, hasil kajian pengekspresan gen menunjukkan tindak balas dua peringkat terhadap stres nitrogen; i) pengumpulan karbohidrat, degradasi protein plastid, dan biosintesis asid amino, dan ii) pengumpulan lipid, degradasi karbohidrat, dan kerosakan DNA. Kesimpulannya, penghasilan stok suapan bahan bakar bio daripada biojisim mikroalga boleh dilaksanakan. Stres nitrogen mencetuskan pengumpulan karbohidrat yang tinggi sebelum karbon diagihkan kepada triasilgliserol (TAG) menggunakan dua tapak jalan berbeza; sintesis TAG kloroplastik dan metabolisme gliserolipid. Implikasi kajian ini boleh dikaitkan dengan potensi pembangunan stok suapan bahan bakar bio daripada biojisim mikroalga untuk aplikasi masa hadapan dan memberikan pemahaman tentang mekanisme pengagihan karbon oleh *C. vulgaris* UPSI-JRM01 dalam keadaan stres nitrogen.

## TABLE OFCONTENTS

## LIST OF TABLES

# LIST OF FIGURES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| CCD | Central Composite Design |
| CCM | $CO_2$ concentrating mechanism |
| CFPP | Cold filter plugging point |
| CN | Cetane number |
| DU | Degree of unsaturation |
| FAME | Fatty acid methyl esters |
| FP | Flash point |
| HBP | High biomass productivity |
| HHV | High heating value |
| HLY | High lipid yield |
| IV | Iodine value |
| KEGG | Kyoto Encyclopaedia of Genes and Genomes |
| KV | Kinematic velocity |
| LCSF | Long-chain saturation factor |
| MUFA | Mono-unsaturated fatty acid |
| N+ | Nitrogen sufficient condition |
| N- | Nitrogen stress condition |
| NCBI | National Centre for Biotechnology Information |
| OD | Optical density |
| PBD | Plackett-Burman Design |
| PPi | Inorganic pyrophosphate |
| PUFA | Poly-unsaturated fatty acid |
| qRT-PCR | Quantitative real time polymerase chain reaction |

| | |
|---|---|
| RNAseq | RNA sequencing |
| RSM | Response Surface Methodology |
| SV | Saponification value |
| SFA | Saturated fatty acid |
| SV | Saponification value |
| TAG | Triacylglycerol |
| UFA | Unsaturated fatty acid |

# LIST OF APPENDIXES

# LIST OF CONFERENCE AND PUBLICATION

**Conference:**

Nordin, N., Yusof, N., Md Nadzir, S., Mohd Yusoff, M. Z., & Hassan, M. A. Optimisation of photoautotrophic cultural conditions for high biomass and lipid production of *Chlorella vulgaris. Paper presented at AFOB-MC International Symposium 2018*, Pullman Hotel and Resorts Kuching, Sarawak. Malaysia: AFOBMCIS 2018.

**Publication 1**:

Nordin, N., Yusof, N., Md Nadzir, S., Mohd Yusoff, M. Z., & Hassan, M. A. (2019). Effect of photo-autotrophic cultural conditions on the biomass productivity and composition of *Chlorella vulgaris. Biofuels.* DOI: 10.1080/17597269.2019.1652787

**Publication 2:**

Nordin, N., Samsudin, S., & Yusof, N. (2019). Isolation and identification of microalgae from high nitrate landfill leachate. *Jurnal Teknologi*, *81*(5), 1–7. DOI: 10.11113/jt.v81.1358

**Publication 3:**

Nordin, N., Yusof, N., Maeda, T., Mustapha, N. A., Mohd Yusoff, M. Z., Raja Khairuddin, R. F. (2020). Mechanism of carbon partitioning towards starch and triacylglycerol in *Chlorella vulgaris* under nitrogen stress through whole-transcriptome analysis. *Biomass and Bioenergy*. 138, 105600. DOI: 10.1016/j.biombioe.2020.105600

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of the Study

The world energy demand for industrial, transportation and buildings is continuously increasing. The major source of energy was currently supplied in the form of non-renewable fossil fuel such as coal, oil and natural gas. However, the exploration of new source of fossil fuel and its utilisation contributes to a significant environmental impact and climate changes (Ribeiro, da Silva, Mata, & Martins, 2015). Moreover, the world has witnessing a gradual increase in petroleum prices over the past few decades due to political conflicts and exhaustion of oil reservoir (Farooq, Suh, Park, & Yang, 2015). Consequently, these concerns had led to an increase of interest in developing alternative energy sources that are sustainable, economically competitive and environmentally friendly. Thus, renewable energy sources particularly biofuel being the main focus of energy field.

The development of biofuel production has been established for four generations. The first generation biofuels such as biodiesel and bioethanol was produced from food crops (i. e. corn, wheat and vegetable oil), which arisen a disagreement of resources competition between fuel and food uses (Ribeiro et al., 2015). Therefore, the second generation biofuels were produced from non-food sources such as waste cooking oil, forestry and agricultural residual biomass. Even though the second generation biofuel was well-established and commercially produced, it could become unsustainable because of limited feedstock sources and available land conflict (Enamala et al., 2018). Hence, due to several advantages, the third and fourth generation biofuels were produced from microalgae and genetically modified microalgae biomass, respectively (Abdullah et al., 2019). These advantages include the high production yield, reduction of the greenhouse gasses ($CO_2$) through carbon fixation, and comparatively easy processing. In addition, microalgae can be massively produce in wide variety of water sources (i. e. fresh, brackish, wastewater and seawater) and in uncultivable desiccated land or marginal farmland (Chen, Zhao, & Qi, 2015). Therefore, competition with food crops production could be avoided.

Microalgae with rapid biomass production and high oil content is among promising oil producing alternative for fossil fuels and oil based crops (Sibi, Shetty, & Mokashi, 2016). They become the desired candidates for a sustainable source of biodiesel as they have the ability to synthesize and accumulate substantial amount of lipid in their cells in the form of triacylglycerol (TAG). Earlier research has shown that microalgae are 20 – 40 times more productive than terrestrial crops (Li et al., 2011), and some of the microalgae could accumulate lipid up to 80% of its dry biomass (Spolaore, Joannis-Cassan, Duran, & Isambert, 2006). Apart from lipid, microalgae also accumulate significant amount of carbohydrate (starch) as a major cell's carbon and energy reservoir. It is well understood that the variations in the environmental and cultural condition attributed to the changes of these energy storage compounds in microalgae. For instance, stressful cultural conditions could change the carbon uptake for starch synthesis and cell's proliferation to TAG synthesis for long-term high energy storage (Sibi et al., 2016). Therefore, deeper understanding regarding the effect of various cultural condition (i. e. temperature, pH, light intensity, nitrogen and carbon supplementation) to the microalgae growth, composition and fixed carbon allocation are crucial for the development of effective and efficient microalgae biomass feedstock generation for biofuel production.

Among the factors that highly influence the biomolecules composition of microalgae is the implementation of nitrogen stress (Jia et al., 2015). In this context, nitrogen stress refers to a condition where the assimilable nitrogen sources such as urea, ammonium ($NH_4^+$), nitrite ($NO_2^-$) and nitrate ($NO_3^-$) are completely unavailable for cellular growth and basic metabolic functioning (Allen, DiRusso, & Black, 2015). Nitrogen stress was often associated with the increase of lipid accumulation. However, the overall biomass productivity was declined (Adams, Godfrey, Wahlen, Seefeldt, & Bugbee, 2013). Nitrogen is a major element in the composition of cell structure, chlorophyll, enzyme, protein and amino acid, therefore its absent substantially affects the synthesis of the afore mentioned components, and consequently inhibited microalgae growth. When the nitrogen required for cellular proliferation is absent, the excessive energy and fixed carbon through photosynthesis is channelled towards the synthesis of energy storage compounds. As nitrogen stress is believed affects the carbon partitioning towards carbohydrate and lipid, it is necessary to understand its mechanism at both cellular and molecular levels.

Several studies on the gene expression in carbohydrate and lipid metabolisms of *Chlorella* sp. under nitrogen stress have been initiated (Ikaran, Suárez-Alvarez, Urreta, & Castañón, 2015; Jiang, Li, Wang, Sun, & Liu, 2019). However, these studies were limited only to few targeted genes analysis with incomplete information obtained to elucidate the complex mechanism of several pathways in protein/ amino acid metabolism, nitrogen metabolism, photosynthesis, carbon fixation and lipid metabolism. A model of carbon partitioning towards TAG in microalgae species *Nannochloropsis oceanica* was already proposed (Jia et al., 2015). However, this proposed model was also based on the results from several targeted genes and did not described the consequences of nitrogen stress to the nitrogen containing compounds (i. e. enzyme, protein, DNA and chlorophyll). In addition, unlike *C. vulgaris*, *N. oceanica* generates β-1,3-glucan but not starch as storage carbohydrates, thus further research is recommended to discover the diversity and evolution of carbon partitioning mechanism in microalgae.

Therefore, in the present research, the metabolic changes of carbohydrate, lipid and protein in *C. vulgaris* UPSI-JRM01 cultured under different photo-autotrophic cultural conditions including nitrogen stress were investigated. In addition, the mechanism of carbon partitioning into carbohydrate, lipid and protein in *C. vulgaris* UPSI-JRM01 under nitrogen stress were also explored by performing the whole transcriptome analysis to investigate the differential gene expression. By doing that, all the genes regulated during nitrogen stress could be identified and the pathways involved in the carbohydrate metabolism, lipid metabolism, protein/ amino acid metabolism, nitrogen metabolism, photosynthesis and carbon fixation could be determined. This study could entail fundamental knowledge particularly on metabolic changes and metabolic pathways involved in carbon partitioning mechanism under nitrogen stress in microalgae. These findings could be prominently important to advance biofuels production towards renewable energy consumption.

## 1.2 Problem Statement and Research Gap

### 1.2.1 Depletion of Fossil Fuel and the Potential of Microalgae as a Sustainable Energy in the Future

The rate of world's energy consumption of non-renewable fossil fuels exceeds the rate of energy generation (Farooq et al., 2015). Fossil fuel combustion leads to emissions of $CO_2$, $SO_2$, $NO_x$, CO, particulate matter and volatile organic compounds which contributes to atmospheric pollution (Tale, Ghosh, Kapadnis, & Kale, 2014). Consequently, the concerns regarding the low availability and dwindling reserve of fossil fuels resources and the environmental impacts associated with its usage were intensified. Hence, there is a need for renewable, environmental friendly and economically sustainable source of energy. In this context, microalgae, in particularly *C. vulgaris* UPSI-JRM01 is seen as potential energy resource for biodiesel production from their biomass.

The interest in the production of microalgae as feedstock for biofuels is increasing due to their high oil content and rapid biomass production, which are the desired criteria for biofuel production. In Malaysia, study of local microalgae species for alternative biofuel production is still scarce. Moreover, worldwide, there are limited success of microalgae biofuel productions that are economic and energy efficient. Therefore, further study is necessary to address the issues concerning the capability of biofuel derived from microalgae biomass as sustainable energy in the future by manipulating several growth factors which influence the composition of produced microalgae biomass.

### 1.2.2 Incomplete Information on the Metabolic Changes of Carbohydrate, Lipid and Protein in Microalgae under Nitrogen Stress

Understanding the carbon partitioning mechanism of microalgae towards lipid and carbohydrate is significant for biofuels strain development and designing cultivation strategies. Although lipid and carbohydrate biosynthesis have been respectively investigated in various microalgae strains (Griffiths, Van Hille, & Harrison, 2014; Jia et al., 2015), less attention has been paid to the combination of these two macromolecules production with protein anabolism and catabolism in response to nitrogen stress. To date, the key points of interactions between nitrogen stress and the metabolic changes of carbohydrate, lipid and protein were remains unclear and need furtherance. The carbohydrate, lipid and protein metabolisms are competitive since they scavenge equivalent photosynthetically fixed carbon as a common precursor for their synthesis (Ikaran et al., 2015). However, in the absent of nitrogen, the cell was unable to synthesis protein, causing the fixed carbon to be partitioned towards carbohydrate and/or lipid. Therefore, further information on the metabolic changes involved in the biosynthesis of these three macromolecules (carbohydrate, lipid and protein) under nitrogen stress is imperative.

### 1.2.3 Limited Information on the Genes Expressed, Metabolic Pathways and Carbon Partitioning Mechanism in Microalgae under Nitrogen Stress

The interaction between the metabolic pathways and mechanisms controlling the biosynthesis of lipid and carbohydrate in microalgae has been widely investigated (Murray, Shields, Garcia, & Healy, 2012). However, less attention has been paid to the combination of these two macromolecules production with the genes expressed in carbohydrate metabolism, lipid metabolism, protein/ amino acid metabolism and nitrogen metabolism in response to nitrogen stress. Previous studies have examined temporal changes in the expression of several target genes under nitrogen stress (Fan, Cui, Wan, Wang, & Li, 2014; Ikaran et al., 2015).

The existing studies on the gene expression in carbon and lipid metabolisms of *Chlorella* were limited only to few target genes analysis such as nitrate reductase (NR), ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo), NADP-dependent malic enzyme (ME), acetyl-coA carboxylase (aCCA) and phosphoenolpyruvate carboxylase (PEPcase). Even though these reports contribute to the understanding of the adaptive mechanisms of microalgae from physiological and molecular point of view, the data provided is still incomplete and unable to elucidate the complex mechanism of carbon partitioning involved due to several target genes analysed. Therefore, more comprehensive study is required to identify the expression level of various genes and metabolic pathway involved in carbohydrate metabolism, lipid metabolism, protein/ amino acid metabolism and nitrogen metabolism in order to propose a model of carbon partitioning mechanism in *C. vulgaris* UPSI-JRM01 under nitrogen stress.

Due to the aforementioned issues, an extensive microalgae metabolic pathway analysis of lipid and carbohydrate by using Kyoto KEGG pathway database was conducted. The KEGG pathways provide database including on genes, transcription, translation, protein and various metabolic pathways including glycolysis, TCA cycle, starch and glucose metabolism, fatty acid metabolism and gluconeogenesis. This approach allows a comprehensive understanding of the molecular mechanisms underlying carbon partitioning in microalgae as it advanced our capacity to explore large-scale data. In this study, genes encoding all known enzymes in the *C. vulgaris* genome were mapped in KEGG pathways by using *Chlorella variabilis* database as a reference, together with $\log_2$-fold differences in mRNA level of genes expression between the nitrogen stress and nitrogen sufficient cultures to elucidate the mechanism of the observed accumulation of carbohydrate and lipid under nitrogen stress. The changes in mRNA levels of the genes involved in the carbohydrate metabolism, lipid metabolism, protein/ amino acid metabolism, nitrogen metabolism, photosynthesis and carbon fixation were analysed in order to obtain further information about physiological, metabolic pathways and carbon partitioning mechanism triggered by nitrogen stress.

## 1.3 Research Objectives

This research consists of three (3) main objectives:

i. To investigate the effect of photo-autotrophic cultural condition (light intensity, temperature, pH, $CO_2$ and $NO_3^-$ concentration) on the maximum biomass productivity and content (carbohydrate, lipid and protein) of *C. vulgaris* UPSI-JRM01 using batch cultivation.

ii. To optimise biomass, lipid and carbohydrate productivities of *C. vulgaris* UPSI-JRM01 for simultaneous biofuel feedstock production.

iii. To elucidate the carbon partitioning mechanism towards starch and triacylglycerol in *C. vulgaris* UPSI-JRM01 under nitrogen stress using whole transcriptome analysis.

## 1.4 Significance of the Study

### 1.4.1 Optimisation of Culture Condition is Necessary Prior to the Utilisation of *C. vulgaris* UPSI-JRM01 Biomass as a Feedstock for Biofuels Production

Optimisation of biomass and lipid biosynthesis in microalgae have been performed by various researchers using statistical approach (Anjos, Fernandes, Vicente, Teixeira, & Dragone, 2013; Karpagam, Raj, Ashokkumar, & Varalakshmi, 2015). Although nitrogen stress is well known to enhance lipid content in many microalgae, previous study showed the response of microalgae in accumulating carbohydrate and lipid when cultured in nitrogen-depletion condition is species specific (Zhu et al. 2014; Ho et al. 2012).

In this study, *C. vulgaris* UPSI-JRM01 was used to obtain a fundamental data on growth, biomass content and their metabolic changes under various environmental conditions including light intensity, temperature, pH, $CO_2$ and $NO_3^-$ concentration. This strain was isolated from Jeram Sanitary Landfill, Selangor, Malaysia and was previously studied for wastewater treatment application and biomass accumulation (Nordin, Yusof, & Samsudin, 2017). Therefore, the optimisation process is necessary prior to the utilization of *C. vulgaris* UPSI-JRM01 biomass as a feedstock for biofuel production. Hence, the results of the most favourable condition for optimum biomass, lipid and carbohydrate productivities can be applied to develop an efficient microalgae cultivation process for biofuel feedstock generation.

### 1.4.2 Improvement of the Carbon Partitioning Mechanism Model of *Chlorella vulgaris* as a Means of Understanding and Exploiting the Links between Carbohydrate, Lipid and Protein Metabolisms at Molecular Level to Improve Biofuel Production

The interaction between nitrogen stress to various metabolic pathways was investigated by generating time-course profiles of carbohydrate, lipid and protein content, lipid body, and fatty acid methyl esters (FAME) of the microalgae cells cultured under nitrogen stress and nitrogen sufficient conditions to highlight its carbon partitioning dynamics. In addition, transcripts levels of the genes in lipid, carbohydrate and protein metabolisms were tracked by combining the pathway available in KEGG database (www.genome.jp/kegg/), with the supports of genes and proteins database in National Center for Biotechnology Information, NCBI (www.ncbi.nlm.nih.gov) and UniProtKB (www.uniprot.org) to reveal the fate of fixed carbon under nitrogen stress (Kanehisa, Sato, Kawashima, Furumichi, & Tanabe, 2016). These lead to the main highlight of this study, which is an improved model of carbon partitioning mechanism in *C. vulgaris* UPSI-JRM01 towards starch and TAG under nitrogen stress. These findings would provide the way of understanding and exploiting the interaction between carbohydrate and lipid metabolisms to enhance biofuel production in *C. vulgaris* and other related microalgae. Moreover, the molecular data on the gene expressed could also provide clues for feasible genetic manipulation to improve biofuel production from microalgae.

### 1.4.3 Development of Alternative Energy Sources that is Renewable, Economically Competitive and Environmentally Friendly

The rapid increase of $CO_2$ concentration in the atmosphere combined with depleted supplies of fossil fuels has led to an increased commercial interest in renewable fuels. *C. vulgaris* have been identified as promising feedstock for industrial scale production of carbon-neutral biodiesel. Carbon-neutral biodiesel resulting in no net emission of $CO_2$ into the atmosphere, as the $CO_2$ released from biodiesel consumption was assimilated during the feedstocks production through photosynthesis. Besides, microalgae cultivation in nitrogen containing wastewater could offer dual application for wastewater treatment and biomass production to the relevant industries. The microalgae-based biofuel has high potential to replace petroleum-based fuel because it is renewable, economically competitive and environmentally friendly (Bhola et al., 2011).

The utilisation of microalgae feedstock has several advantages over traditional feedstock from plant biomass. These photosynthetic organisms are easily adapted to environmental conditions, high photosynthesis efficiency, produce high oil and biomass yields, can be cultivated within non-freshwater sources, can be grown on non-arable land, do not compete with common food resources, and efficient use of water and fertilizers for growth (Jones & Mayfield, 2012; Zhu et al., 2014). Accordingly, this research has a significant contribution to the development of microalgae-derived biofuel that complied with the imposed biodiesel standard (ASTM D6751 and EN14214).

## 1.5 Scope and Limitation of the Study

This study focuses on the enhancement of biomass, lipid and carbohydrate yields and productivities in *C. vulgaris* UPSI-JRM01 for biofuel feedstock production. To achieve that, the effect of photo-autotrophic cultural condition (light intensity, temperature, pH, $CO_2$ and $NO_3^-$ concentration) on the maximum biomass productivity and content was investigated through single factor experiment. The biomass, lipid and carbohydrate productivities were then optimised using statistical approaches of Plackett-Burman Design (PBD) and Central Composite Design (CCD). In addition, the properties of microalgae-derived biodiesel were also evaluated to determine the feasibility and potential of biofuel production from *C. vulgaris* UPSI-JRM01 biomass. In response to the necessity for high carbohydrate and lipid yields, this study was also designed a cultivation of *C. vulgaris* UPSI-JRM01 under nitrogen stress condition. This is imperative to understand the fundamental mechanism lies for carbon partitioning towards starch and TAG under nitrogen stress.

However, this study also had several limitations. The species of microalgae used in this study was limited to the locally isolated microalgae species, *C. vulgaris* UPSI-JRM01. Moreover, the mechanism of carbon partitioning in *C. vulgaris* UPSI-JRM01 was analysed only under nitrogen stress condition as it highly influences TAG and starch accumulation inside the microalgae cells compared to other culture condition. Furthermore, the database for functional annotation of protein sequences available for *C. vulgaris* was scarce. In this study, NCBI, UniProtKB and KEGG databases were used as the main references to determine the homology of protein sequences, protein functions, gene nomenclatures and metabolic pathways involved under nitrogen stress.

## 1.6 Thesis Organisation

The thesis is organised into seven chapters as summarised in Figure 1.1. Chapter 1 begins with an introduction to the background of the study. This chapter describes the problem statement, research gap, research objectives and significance of the study. In addition, this chapter also discuss the scope and limitation of the study.

Chapter 2 presents a literature review of previous studies from relevant sources that contributed to the intellectual progression of the current issues, findings, theories, and debates in the area of present study. This chapter provides conceptual description, organisational pattern and critical evaluation of these works in relation to the study being investigated.

The three research objectives to be achieved in this study are addressed in Chapters 3 – 5. The format of each chapter consists of introduction, methodology, results, discussions and conclusions. Chapter 3 discusses the effect of photo-autotrophic cultural condition on the maximum biomass productivity and content of *C. vulgaris* UPSI-JRM01. Meanwhile, Chapter 4 presents the optimisation of biomass, lipid and carbohydrate productivities in *C. vulgaris* UPSI-JRM01 for simultaneous biofuel feedstock production. In addition, Chapter 5 elucidates the mechanism of carbon partitioning towards starch and triacylglycerol in *C. vulgaris* UPSI-JRM01 under nitrogen stress through whole transcriptome analysis.

Next, Chapter 6 encompasses the general discussions on the overall research findings. It describes and interprets the relationship of each chapter to the other, connects with prior theories, and focuses on the novelty and implication of the study. Chapter 7 then concludes the thesis and provides the recommendations for future study. The references and appendices are cited and attached accordingly at the end of the thesis.

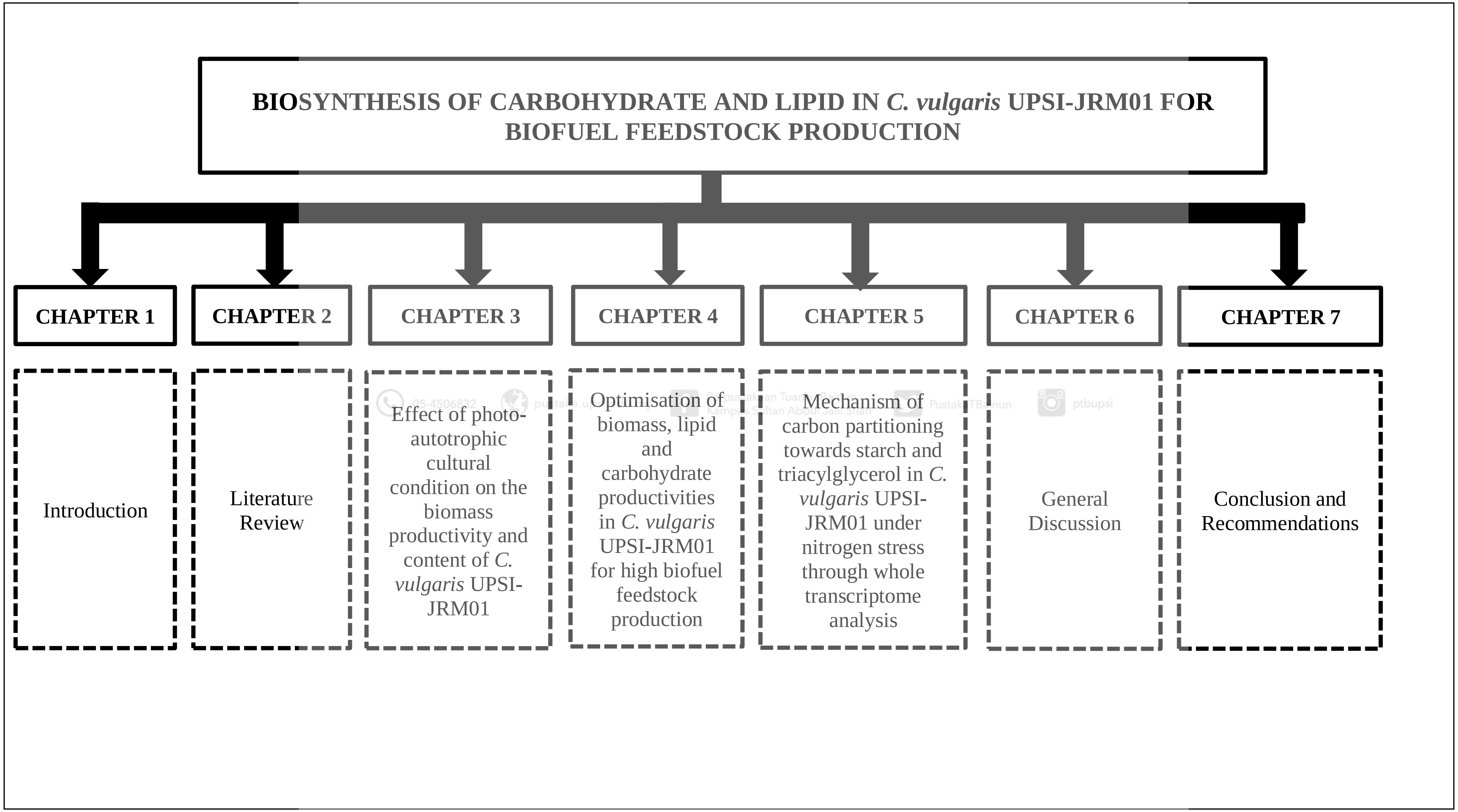

*Figure 1.1.* Summary of the thesis