Production of bio-jet fuel from microalgae

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Production of bio-jet fuel from microalgae

Abstract
The increase in petroleum-based aviation fuel consumption, the decrease in petroleum resources, the fluctuation of the crude oil price, the increase in greenhouse gas emission and the need for energy security are motivating the development of an alternate jet fuel. Bio-jet fuel has to be a drop in fuel, technically and economically feasible, environmentally friendly, greener than jet fuel, produced locally and low gallon per Btu. Bio-jet fuel has been produced by blending petro-based jet fuel with microalgae biodiesel (Fatty Acid Methyl Ester, or simply FAME). Indoor microalgae growth, lipids extraction and transestrification to biodiesel are energy and fresh water intensive and time consuming. In addition, the quality of the biodiesel product and the physical properties of the bio-jet fuel blends are unknown. This work addressed these challenges. Minimizing the energy requirements and making microalgae growth process greener were accomplished by replacing fluorescent lights with light emitting diodes (LEDs). Reducing fresh water footprint in algae growth was accomplished by waste water use. Microalgae biodiesel production time was reduced using the one-step (in-situ transestrification) process. Yields up to 56.82 mg FAME/g dry algae were obtained. Predicted physical properties of in-situ FAME satisfied European and American standards confirming its quality. Lipid triggering by nitrogen deprivation was accomplished in order to increase the FAME production. Bio-jet fuel freezing points and heating values were measured for different jet fuel to biodiesel blend ratios.

Keywords
Engineering, Chemical, Energy, Alternative Energy

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PRODUCTION OF BIO-JET FUEL FROM MICROALGAE

BY

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THESIS

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DEDICATION

To my daughters Yuliana A. Elmoraghy and Youanna A. Elmoraghy
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**NOMENCLATURE**

The following variables, arranged in alphabetic order are used in this thesis. The list includes variable name, units, and a short definition/comment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Definition/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{algae}$</td>
<td>mg algae/L Solution</td>
<td>Algae concentration</td>
</tr>
<tr>
<td>$C_{cal}$</td>
<td>KJ/g</td>
<td>Heat capacity of the calorimeter</td>
</tr>
<tr>
<td>$C_i$</td>
<td>mg/l</td>
<td>Initial microalgae biomass concentration</td>
</tr>
<tr>
<td>$C_f$</td>
<td>mg/l</td>
<td>Final microalgae biomass concentration</td>
</tr>
<tr>
<td>$C_{oil}$</td>
<td>mg oil/L Solution</td>
<td>Oil concentration</td>
</tr>
<tr>
<td>$f$</td>
<td>-</td>
<td>Function that represents any physical property</td>
</tr>
<tr>
<td>$i$</td>
<td>-</td>
<td>Vant Hoff factor</td>
</tr>
<tr>
<td>$I$</td>
<td>$\mu$mol.m$^{-1}$.s$^{-1}$</td>
<td>Light intensity</td>
</tr>
<tr>
<td>$I_0$</td>
<td>$\mu$mol.m$^{-1}$.s$^{-1}$</td>
<td>The original incident intensity</td>
</tr>
<tr>
<td>$I_L$</td>
<td>$\mu$mol.m$^{-1}$.s$^{-1}$</td>
<td>The light intensity at depth L</td>
</tr>
<tr>
<td>$k_{algae}$</td>
<td>day$^{-1}$</td>
<td>Growth rate constant of algae</td>
</tr>
<tr>
<td>$K_f$</td>
<td>K/(mol/kg)</td>
<td>Cryoscopic constant of the solvent</td>
</tr>
<tr>
<td>$k_{oil}$</td>
<td>day$^{-1}$</td>
<td>Growth rate constant of algae</td>
</tr>
<tr>
<td>$K_i$</td>
<td>$\mu$mol.m$^{-1}$.s$^{-1}$</td>
<td>Saturation constant</td>
</tr>
<tr>
<td>$K_2$</td>
<td>$\mu$mol.m$^{-1}$.s$^{-1}$</td>
<td>Inhibition parameter</td>
</tr>
<tr>
<td>$L$</td>
<td>m</td>
<td>Depth</td>
</tr>
<tr>
<td>$m$</td>
<td>mol/kg</td>
<td>Molality</td>
</tr>
<tr>
<td>$m_{fuel}$</td>
<td>g</td>
<td>Mass of the fuel</td>
</tr>
<tr>
<td>$m_i$</td>
<td>-</td>
<td>Mass fraction of fuel i</td>
</tr>
<tr>
<td>$M_i$</td>
<td>g/gmol</td>
<td>Molecular weight of the $i$th FAME</td>
</tr>
<tr>
<td>$N$</td>
<td>-</td>
<td>Number of double bonds in fatty acids</td>
</tr>
<tr>
<td>$q_{fuel}$</td>
<td>KJ/g</td>
<td>Heat released by the combustion of the fuel</td>
</tr>
<tr>
<td>$q_{wire}$</td>
<td>KJ/g</td>
<td>Heat released by the combustion of the wire</td>
</tr>
<tr>
<td>$S_{Blend}$</td>
<td>-</td>
<td>Specific gravity of the blend</td>
</tr>
<tr>
<td>$S_{Gi}$</td>
<td>-</td>
<td>Specific gravity of the fuel $i$</td>
</tr>
<tr>
<td>$t$</td>
<td>day</td>
<td>Time</td>
</tr>
<tr>
<td>$T$</td>
<td>°C</td>
<td>Temperature</td>
</tr>
<tr>
<td>$X$</td>
<td>mg/l</td>
<td>Microalgae concentration at time $t$</td>
</tr>
<tr>
<td>$X_0$</td>
<td>mg/l</td>
<td>Concentration of chlorophyll a or total chlorophyll</td>
</tr>
<tr>
<td>$X_i$</td>
<td>Arbitrary units</td>
<td>Area under the unknown peak, corresponding to $Y_i$</td>
</tr>
<tr>
<td>$X_s$</td>
<td>Arbitrary units</td>
<td>Area under the peak, corresponding to $Y_s$</td>
</tr>
<tr>
<td>( Y_i )</td>
<td>mg FAME/ml</td>
<td>Concentration of the unknown FAME</td>
</tr>
<tr>
<td>( Y_s )</td>
<td>mg FAME/ml</td>
<td>Concentration of the FAME when injected individually</td>
</tr>
<tr>
<td>( z_i )</td>
<td>-</td>
<td>Mass fraction of the ( i )th FAME</td>
</tr>
</tbody>
</table>

Greek Variables

| \( Y \) | \( m^{-1} \) | Turbidity coefficient |
| \( \mu_{\text{max}} \) | day\(^{-1}\) | Maximum microalgae specific growth rate |
| \( \mu \) | day\(^{-1}\) | Specific growth rate |
| \( \rho_i \) | g/ml | Density of the fuel \( i \) or \( i \)th FAME |
| \( \rho_{\text{water}} \) | g/ml | Density of water |
| \( \Phi_i \) | - | Cetane number of the \( i \)th FAME or the fuel |
| \( \nu_i \) | mm\(^2\)/s or cSt | Kinematic viscosity |
| \( \delta_i \) | KJ/g | Higher heating value of the \( i \)th FAME or the fuel |
| \( \Delta T_f \) | K | Difference between freezing point of the mixture and of the pure solvent |
ABSTRACT

PRODUCTION OF BIO-JET FUEL FROM MICROALGAE
BY
MARIAN ELMORAGHY
UNIVERSITY OF NEW HAMPSHIRE, AUGUST 2013

The increase in petroleum-based aviation fuel consumption, the decrease in petroleum resources, the fluctuation of the crude oil price, the increase in greenhouse gas emission and the need for energy security are motivating the development of an alternate jet fuel. Bio-jet fuel has to be a drop in fuel, technically and economically feasible, environmentally friendly, greener than jet fuel, produced locally and low gallon per Btu. Bio-jet fuel has been produced by blending petro-based jet fuel with microalgae biodiesel (Fatty Acid Methyl Ester, or simply FAME). Indoor microalgae growth, lipids extraction and transertrification to biodiesel are energy and fresh water intensive and time consuming. In addition, the quality of the biodiesel product and the physical properties of the bio-jet fuel blends are unknown. This work addressed these challenges. Minimizing the energy requirements and making microalgae growth process greener were accomplished by replacing fluorescent lights with light emitting diodes (LEDs). Reducing fresh water footprint in algae growth was accomplished by waste water use. Microalgae biodiesel production time was reduced using the one-step (in-situ transestrification) process. Yields up to 56.82 mg FAME/g dry algae were obtained. Predicted physical properties of in-situ FAME satisfied European and American standards confirming its quality. Lipid triggering by nitrogen deprivation was accomplished in order to increase the FAME production. Bio-jet fuel freezing points and heating values were measured for different jet fuel to biodiesel blend ratios.
CHAPTER I

INTRODUCTION

1.1 BACKGROUND

The US is one of the world's largest importers of Petroleum oil. The current US annual diesel demand for ground transportation is estimated to be around 70 billion gallons (Elmoraghy et al., 2012). The increase in oil prices and the need to improve the US energy security provides a strong incentive to research renewable fuel sources. One research area is biofuels, expected to create local jobs and offer alternatives to the US reliance on petroleum based fuels (Chisti, 2008). Traditionally, biofuels were produced from corn, soybean, canola, and sugar cane. While these fuel feedstock sources are renewable and are more environmentally friendly than petroleum fuel sources, they have their drawbacks. These crops are supposed to be “food crops” for the US and the rest of the world. According to the 2009 figures from the US Department of Agriculture, roughly one-quarter of all the maize and other grain crops grown in the US are used as feedstock to biofuel that ends up in cars rather than feeding people. Roughly, 50 million tons of US grains (enough to feed 160 million people for one year) were used to make ethanol for cars. In 2009, the 50 million jumped to 90 million tons of US grain. The use of these grains as “energy crops” has led to a highly undesirable increase in food prices. In addition, plant energy crops are a dispersed source of energy requiring large land acreage to produce the required oil feedstock. For example, an acre of soybeans only produces about 60 gallons of biodiesel oil feedstock per year (Ferrentino 2007, Mulumba 2010).
1.2 AVIATION TURBINE FUEL (JET FUEL) NEEDS

Biodiesel seems to be an adequate replacement for diesel fuel used in ground transportation. An important challenge is to find an adequate replacement for aviation turbine fuels, or simply jet fuels. The main use of aviation turbine fuels is to power jet and turbo engine aircraft.

Biodiesel alone is not suitable as a jet fuel. There are very specific requirement that the jet fuel must meet, e.g., the energy density, and the low temperature fuel properties for any alternative option are quite important. Biodiesel tends to freeze at the low temperatures that airplanes are likely to encounter at high altitude cruising.

1.3 JET FUEL GRADES

There are currently two main grades of turbine fuel in use in civil commercial aviation: Jet A-1 and Jet A, both are kerosene type fuels. There is a third grade of jet fuel, Jet B, which is a wide cut kerosene (a blend of gasoline and kerosene) but it is rarely used except in very cold climates. Light jet fuels (kerosene) are refined from distillation of crude oil.

Military jet fuel JP-4 is Jet B chemically enhanced with antioxidants, dispersants, or corrosion inhibitors. Similarly JP-5 is chemically enhanced kerosene and JP-8 is chemically enhanced Jet A-1. The enhancement is needed to meet the requirements for a specific application. Table 1.1 shows different types of civil/commercial and military Jet fuels.
Table 1.1: Different types of commercial and military jet fuels
http://www.csgnetwork.com/jetfuel.html

<table>
<thead>
<tr>
<th>Commercial Jet Fuels</th>
<th>Military Jet Fuels</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Jet A-1</strong></td>
<td></td>
</tr>
<tr>
<td>Kerosene grade suitable for most turbine engine aircraft.</td>
<td>JP-4</td>
</tr>
<tr>
<td>Flash point above 38°C (100°F)</td>
<td>The military equivalent of Jet B with the addition of corrosion inhibitor and anti-icing additives.</td>
</tr>
<tr>
<td>Freezing point maximum of (-47°C)</td>
<td>Meets certain of U.S. military specification.</td>
</tr>
<tr>
<td>Net heat of combustion minimum of (42.8MJ/kg)</td>
<td></td>
</tr>
</tbody>
</table>

| **JET A**            |                     |
| Kerosene type of fuel. | JP-5               |
| Flash point above 38°C (100°F) | High flash point kerosene. |
| Freeze point maximum (-40°C) | Meets the requirements of the U.S. and British Specification |

| **JET B**            |                     |
| Distillate covering the naphtha and kerosene fractions. | JP-8               |
| Freeze point maximum of (-50°C) | The military equivalent of Jet A-1 with the addition of corrosion inhibitor and anti-icing additives. |
| Net heat of combustion minimum of (42.8MJ/kg) | Meets the requirements of the U.S. Military Specification. |
| Higher flammability |                     |
| Significant demand in very cold climates. |                     |

1.4 BIO- JET FUEL AS A RENEWABLE JET FUEL

The airline industry is faced with the challenges of increasing petroleum oil prices and persistent oil dependency and the deteriorating climate due to greenhouse gas emission. The industry desires a sustainable fuel that is less dependent on and greener than petroleum-based jet fuel and would not require high volume per unit energy. The replacement fuel should be a “drop-in” fuel, i.e., easily blended with or directly replacing jet fuel, would not require changes to aircraft design and would not detriment airplane maneuverability.
Several alternative jet fuel options are available. These include synthetic fuels, biodiesel-based bio-jet fuel, and cryogenic fuels (e.g., liquid hydrogen). These fuels must be studied and their advantages and disadvantages as a "drop-in" fuel clearly understood. Cryogenic fuels (e.g., liquid hydrogen) are expected to be a long-term solution for aviation fuels, but these will require design changes and technological advances to the airplanes engines. The Pew Center (2010) study suggests that the production of biofuels is greener than the production of synthetic fuels. Consequently, biofuels, e.g., blends of biodiesel are more desirable replacement for jet fuel.

The focus of this study is the jet fuel based on microalgae biodiesel, or simply bio-jet fuel. It could reduce flight-related greenhouse-gas emissions by over 60 percent compared to fossil fuel based jet fuel. Compared to other fuels, bio-jet fuel has a low gallon per Btu. In addition, it can be blended with petroleum-based jet fuel.

1.5 BIO-JET FUEL FROM MICROALGAE

There are three routes to produce bio-jet fuel from microalgae. Figure 1.1 shows these three routes.
1.5.1 BIO DERIVED SYNTHETIC PARAFFINIC KEROSENE (BIO-SPK)

The first route involves using algae oil to produce bio-SPK (Bio derived Synthetic Paraffinic Kerosene) by cracking and hydro processing. This can be used for kerosene-type fuels include Jet A, Jet A-1, JP-5, and JP-8.

The growing of algae to make jet fuel is a promising but still an emerging technology. Companies working on algae jet fuel include Solazyme, Honeywell UOP, Solena, Sapphire Energy, Imperium Renewables, and Aquaflow Bionomic Corporation. Universities working on algae jet fuel are Arizona State University, Cranfield University. Major investors for algae based SPK are Boeing, Honeywell/UOP, Air New Zealand (ANZ), Continental Airlines (CAL), Japan Airlines (JAL), and General Electric.
1.5.2 FISHER-TROPSCH SYNTHETIC PARAFFINIC KEROSENE (FT-SPK)

The second route involves processing solid biomass using pyrolysis to produce pyrolysis oil or gasification to produce a syngas, which is then possessed into FT-SPK (Fisher-Tropsch Synthetic Paraffinic Kerosene).

1.5.3 BLENDING ALGAE BIODIESEL WITH KEROSENE TO PRODUCE BIOFUEL

The third route to produce bio-jet fuel is blending algae biodiesel with kerosene. This route involves algae growth, harvesting, oil extraction, and transestrification (or in-situ process) to produce microalgae biodiesel. Table 1.2 lists the properties of biodiesel and petroleum-based jet fuel. Microalgae biodiesel will be blended with conventional petroleum-derived jet fuel to provide bio-jet fuel with the necessary specification properties.

Table 1.2: Comparison of biodiesel vs. conventional jet fuel, (Chevron, 2010)

<table>
<thead>
<tr>
<th>Fuel Property</th>
<th>Biodiesel</th>
<th>Petroleum-based Jet Fuel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flash Point, C</td>
<td>100</td>
<td>40 - 45</td>
</tr>
<tr>
<td>Kinematic Viscosity at 40 C, cSt</td>
<td>4.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Net Heat of Combustion, MJ/kg</td>
<td>36-39</td>
<td>43.2</td>
</tr>
<tr>
<td>Specific gravity, 15 C</td>
<td>0.87 - 0.89</td>
<td>0.80</td>
</tr>
<tr>
<td>Freezing point, C</td>
<td>About 0</td>
<td>&lt; -40</td>
</tr>
<tr>
<td>Approximate number of carbon atoms</td>
<td>C16 to C22</td>
<td>C8 to C16</td>
</tr>
<tr>
<td>Sulfur, wt%</td>
<td>&lt;0.05</td>
<td>0.05 - 0.15</td>
</tr>
</tbody>
</table>
1.6 BIODIESEL

Biodiesel is comparable to conventional petroleum diesel in energy density, Cetane number, heat of vaporization, and stoichiometric air/fuel ratio (Rajan et al., 2010). Moreover, biodiesel is renewable, biodegradable, non-toxic, sulfur free, and a carbon-neutral fuel source. Other advantages of biodiesel include superior lubricant and solvent properties, lower emissions of harmful chemicals, ease of storage and transportation, and can be used in diesel engines without any modification of the engine.

From an environmental and safety point of view, biodegradability and toxicity are important properties of a fuel. Peterson et al., 2005 demonstrated that biodiesel degrades approximately 4 times as fast as conventional diesel in aquatic environments. Biodiesel was found to be just as biodegradable as simple sugar. They also showed that biodiesel is not only considerably less toxic than diesel fuel, but also up to 89 times less toxic than table salt, making it a safer and more environmentally friendly alternative fuel.

Cetane number is a measure of combustion quality of a diesel engine during compression ignition. Higher-Cetane fuel usually causes an engine to run more smoothly and quietly. In the US, most states require a minimum diesel fuel Cetane number of 40 (California requires 53). The typical range is 42-45. Biodiesel Cetane numbers range is 46 to 52 depending on the feedstock used, (Encinar et al., 2005). Hence, biodiesel improves the performance of diesel engines.

Biodiesel is a clean burning fuel that does not contribute to the net increase of carbon monoxide. In addition, the study by the National Renewable Energy Laboratory (NREL) showed that overall CO₂ emissions were reduced by 78% when compared to
conventional diesel. Biodiesel use also reduces the emissions of sulfur dioxide, particulate matter and unburned hydrocarbons. Burning biodiesel fuel has slightly higher NO\textsubscript{x} emissions than conventional diesel, Choi and Reitz, 1999. These NO\textsubscript{x} emissions can be eliminated with the use of proper additives, e.g., antioxidants. McCormick et al, 2003.

Biodiesel provides an effective, sustainable fuel with many desirable properties. The major disadvantage is the production cost (US DOE, 2013), driven by the high feedstock prices.

Traditionally, biodiesel is produced from oleaginous crops. These plants are cultivated essentially for oil production, for either nutritional or industrial consumption. Examples include oilseed rape, sunflower, corn, olive, soya-bean and flax. The use of these grains as “energy crops” has led to a highly undesirable increase in food prices and food riots. Refined oils, such as soybean and rapeseed oil, are expensive and generally account from 60% to 80% of the total cost of biodiesel. Due to these high feedstock prices, without government-grant tax breaks, biodiesel is not currently cost-competitive with conventional diesel. More recently, some less refined and less expensive feedstocks have been tested for use in biodiesel production so that it may better compete with conventional diesel. The most promising feedstock is microalgae. The average biodiesel production from microalgae can be 10 to 20 times higher than the production from oleaginous seeds, such as rapeseed, soybean, sunflower, and palm (Gouveia et al., 2009). Table 1.3 shows a comparison of some sources of biodiesel.
Table 1.3 Comparison of biodiesel oil feedstock yield (liter per hectare) from several sources (L/ha<sup>1</sup> from Gouveia et al., 2009), (L.m<sup>2</sup>.yr<sup>-1</sup>) from Melinda et al., 2011, Mata et al., 2010 and Sazdanoff, 2006)

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Oil Yield (L/ha&lt;sup&gt;1&lt;/sup&gt;)</th>
<th>Yield (L.m&lt;sup&gt;2&lt;/sup&gt;.yr&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>172</td>
<td></td>
</tr>
<tr>
<td>Soybean</td>
<td>446</td>
<td>0.04</td>
</tr>
<tr>
<td>Canola</td>
<td>1,190</td>
<td>0.12</td>
</tr>
<tr>
<td>Jatropha</td>
<td>1,892</td>
<td>0.19</td>
</tr>
<tr>
<td>Palm</td>
<td>5,950</td>
<td>0.54</td>
</tr>
<tr>
<td>Microalgae, 30% oil (by weight) in biomass</td>
<td>58,700</td>
<td>4.7 to 14</td>
</tr>
<tr>
<td>Microalgae, 70% oil (by weight) in biomass</td>
<td>136,900</td>
<td></td>
</tr>
</tbody>
</table>

1.6.1 MICROALGAE BIODIESEL

Biodiesel derived from corn, soybean, rapeseed, Jatropha, and oil palm are available in the market. Estimates are that the global biodiesel market will reach 37 billion gallons by 2016, with an average annual growth of 42%. Europe is the major biodiesel market followed by US. In order to meet these rapid production capacity of biodiesel, other oil sources especially non-edible oil should be used, e.g., Jatropha curcas (Farag, 2009, Tewfik et al, 2012).

Microalgae oil is the only renewable source that has the potential to displace petroleum-derived transport fuels diesel fuel completely without the argument “food for fuel” (Gouveia et al., 2009). This is obtained by growing single celled high lipid microalgae and extracting their oil/natural lipids. Some microalgae have high lipid content, making these microalgae suitable for lipid/oil production. Table 1.4 lists some of microalgae species and their content.
Table 1.4: lipid content of some microalgae (%dry matter) (Gouveia et al., 2009)

<table>
<thead>
<tr>
<th>Species</th>
<th>Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scenedesmus obliquus</td>
<td>11-22</td>
</tr>
<tr>
<td>Scenedesmus dimorphuus</td>
<td>6-7</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>6-7</td>
</tr>
<tr>
<td>Chlorella emersonii</td>
<td>14-40</td>
</tr>
<tr>
<td>Chlorella protothecoides</td>
<td>23</td>
</tr>
<tr>
<td>Chlorella sorokiana</td>
<td>22</td>
</tr>
<tr>
<td>Chlorella minutissima</td>
<td>57</td>
</tr>
<tr>
<td>Dunaliella bioculata</td>
<td>8</td>
</tr>
<tr>
<td>Dunaliella salina</td>
<td>14-20</td>
</tr>
<tr>
<td>Neochloris oleoabundans</td>
<td>35-65</td>
</tr>
<tr>
<td>Spirulina maxima</td>
<td>4-9</td>
</tr>
</tbody>
</table>

The triacylglycerides (TAGs) in the microalgae neutral lipids are converted to biodiesel FAME (Fatty Acid Methyl Ester) or VOME (vegetable Oil Methyl Ester) by the transestrification process, which takes place between the TAGs and methanol in presence of catalyst (Wilson et al., 2012). This catalyst could be acid or alkali. The highest degrees of oil conversion into methyl esters, Biodiesel FAME, can be obtained using alkaline catalyst: KOH and NaOH (Mulumba, 2010 and 2012). Potassium and sodium hydroxides are good and inexpensive catalysts (Wcislo, 2008). In this transestrification reaction, an ester and an alcohol, e.g., methanol reacts to form a different ester. The three fatty acid chains (RiCOO-) connected to the glycerol backbone are broken at their ester bond and react with the alcohol to form alkyl esters and a glycerol molecule Babcock et al., as shown in reaction (1.1).
Some microalgae have a convenient fatty acids profile and an unsaponifiable fraction allowing a biodiesel production with high oxidation stability. The physical and fuel properties of biodiesel from micro-algal oil in general (e.g., density, viscosity, acid value, heating value, etc.) are comparable to those of fuel diesel (Gouveia et al., 2009).

1.7 MICROALGAE

Based on their energy source microalgae species are classified into three types, autotrophic, heterotrophic and mixotrophic. Autotrophic species are photosynthetic similar to plants. Heterotrophic species get their energy from organic carbon compounds (e.g., sugars) similar to yeast, bacteria and animals. Mixotrophic species can use sunlight or organic carbon, whichever is available. Autotrophic microalgae can be a suitable alternative biodiesel feedstock since algae are very efficient biological producer of oil and a versatile biomass source. They may soon be one of the earth’s most important renewable fuel crops. This is due to the higher photosynthetic efficiency, higher biomass productivities, a faster growth rate than higher plants, highest CO$_2$ fixation and O$_2$ production and growing in liquid medium, which can be handled easily. Microalgae can be grown in variable climates, hot sunny climates (Wilson et al, 2012) and non-arable
land including marginal areas unsuitable for agricultural purposes (e.g. desert and seashore lands). Moreover, Algae can be grown in non-potable water using far less fresh water than traditional crops especially when closed systems (photobioreactors) are used. The production of one liter of biodiesel from oil crops requires around 3,000 liters of water over a period of several months. On the other hand, 1 liter of biodiesel from microalgae with 50% lipid content needs 10 to 20 liters only (Schlagermann et al., 2012). In addition, Algae production is not seasonal and can be harvested daily.

The ability of algae to fix CO$_2$ can also be an interesting method of decarbonizing power plants exhaust gases. One kg of dry algal biomass utilizes about 1.83 kg of CO$_2$ (Chisti Y. et al., 2007, 2008) while growing. Using higher lipids production microalgae will reduce greenhouse gases and consequently produce higher biodiesel yield. (Gouveia et al., 2009).

### 1.8 MICROALGAE GROWTH

#### 1.8.1 MICROALGAE GROWTH CYCLE

Algae growth can be broken up into four separate phases, lag, exponential, stationary, and lysis phase. The lag phase of growth occurs after the cells have been inoculated into the nutrient medium. During this phase of growth the cells are getting adjusted to their new nutrient medium and very little doubling occurs. Once the algae cells are acclimated to these new growth conditions they enter what is known as the exponential growth phase. During this phase of growth the maximum cell doubling is observed; the cells double at a constant rate. At the end of the exponential growth phase, the maximum cell
concentration enters the stationary phase of growth. During the stationary phase of growth minimal cell doubling occurs. In this phase of growth, the cells have used up most of the nutrients available to them. Once the nutrients are used up, the cells will enter the lysis phase. During this phase, the cell density begins to drop as the cells die from nutrient starvation. If the algae cells are to be harvested so that a maximum biomass yield can be obtained then they should be harvested around the time they enter the stationary growth phase (Elmoraghy et al., 2012). Figure 1.2 presents the growth cycle and lipid accumulation of Chlorella vulgaris by Mallick et al., 2012. They showed that Chlorella vulgaris growth increased steadily with a lag of 3 days followed by the logarithmic phase, and attained the stationary phase on day 18. Maximum accumulation of lipid was observed at the stationary phase (96.3 mg L⁻¹, 9.2% dry cell weight). After 24 days the lipid showed a declined trend.

![Figure 1.2 Biomass production and Lipid accumulation in Chlorella vulgaris (Mallick et al., 2012)](image)
1.8.2 PHOTOSYNTHESIS PROCESS

Autotrophic microalgae growth use photosynthesis process as a key component of their survival, whereby they convert solar radiation and CO2 absorbed into glucose, which is then used in respiration to produce energy to support growth (Brennan et al., 2010). Reaction (1.2) is the summary formula for photosynthesis, which describes how light energy, usually 8 photons, is transformed into glucose, $C_6H_{12}O_6$.

$$\text{Light Energy (8 photons)} + 6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow C_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \quad \text{Reaction (1.2)}$$

This glucose can then be consumed produce chemical energy in the process of respiration.

$$C_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} \quad \text{Chemical Energy} \quad \text{Reaction (1.3)}$$

This respiration chemical energy is used in the microalgae cell to build chemical bonds in the form of energy-dense lipids. Photosynthesis, lipids extraction and transestrification transfer the energy present in the light that hits the algae into the chemical energy available in biodiesel fuel.

1.8.3 PHOTOSYNTHESIS LIGHT SOURCE FOR ALGAE GROWTH

Sunlight is a free and widely available source of light for autotrophic algae growth. The limitations of available sunlight are due to seasonal and regional variations. The use of artificial lighting sources allows for algae growth at any time of the year or in any region. Moreover, the electric supply for the artificial lighting is derived from fossil fuels. Therefore, there is a conflict with the aims of developing a price competitive fuel and decreasing the carbon footprint. It is therefore important to focus on lighting that will
produce useful spectra for algae growth. It is also important to understand the absorption spectra of major algal accessory pigments present in various quantities in different algal groups. The two primary pigments found in the chloroplast are chlorophyll a and chlorophyll b. There are also several other accessory pigments that play a small role in photosynthesis (carotenoids for example, are present in chlorella species). Chlorophyll a and b absorb light differently as shown in Figure 1.3

![Figure 1.3 light absorption by pigments (UNH Center for Freshwater Biology)](image)

These pigments are NOT present in equal proportions in a chloroplast. If these light absorption curves are weighted by content in the cell, a photosynthetic action spectrum of the chlorophyll can be produced, as shown in Figure 1.4. The figure shows that the relative photosynthetic rate of the algae peaks at blue/violet and red colors. These explain why red & blue lights are so effective in algae growth at optically thin cultures. Similarly, Green and yellow lights (500-600nm) are not well absorbed in optically thin cultures, as indicated by the dip in the graph. This is because none of the pigments absorbs green or yellow light very well (see Figure 1.3). Several studies reported that the optimal
wavelength condition of microalgae could vary from species to species (Chen et al., 2011).

![Photosynthetic Action Spectrum](image)

Figure 1.4 Photosynthetic Action Spectrum (UNH Center for Freshwater Biology)

1.8.4 LIGHT EMITTING DIODES (LEDs)

The cultivation of microalgae requires effective illumination to reduce the production cost of the algae oil and the resulting bio-jet fuel. Sunlight is basically free, but its intensity changes between day and night fluctuates and between summer and winter. These changes limit the available solar energy intensity. This necessitates the use of inexpensive and reliable electric illuminating devices that convert energy to light with high efficiency, generate little or no heat, and emit light only at the wavelengths at which the relative photosynthetic rate of the algae (Figure 1.4) is highest.

Among the light sources currently available, light-emitting diodes (LEDs) are the only ones that meet the foregoing criteria. There is available a wide variety of LEDs emitting from red to purple light. LEDs have narrow light emission spectra between 20
and 30 nm, which can be matched with the photosynthetic needs. For example, the absorption wavelength of blue LED and red LED are around 450-470 nm and 645-665 nm, respectively (Figure 1.4).

1.9 PHOTOBIOREACTORS

There are two main types of algae cultivation systems: open pond and closed photobioreactors.

1.9.1 OPEN PONDS

Open pond production systems can be categorized into natural waters (lakes, lagoons, and ponds) and artificial system. Raceway is the most commonly used artificial system. They are typically made of a closed loop, oval shaped recirculation channels (Figure 1.5), with mixing and circulation required to stabilize algae growth and productivity. Paddle wheel is operated continuously to prevent sedimentation. Open ponds are the cheaper method of large-scale algae biomass production compared to closed photobioreactor. Open ponds do not compete with agricultural crops for land, since they can be built on non-arable land. They also have lower energy input requirement, and regular maintenance and cleaning are easier. On the other hand, open ponds are threatened by contamination. They are less efficient than closed photobioreactor with respect to biomass productivity. This is due to evaporation losses, temperature fluctuation in the growth media, CO₂ deficiencies, inefficient mixing, and light limitation (Brennan et al., 2010).
1.9.2 CLOSED PHOTOBIOREACTORS

A photobioreactor (PBR) is a closed bioreactor that incorporates some type of light source to provide photonic energy input into the reactor. The PBR overcomes algae contamination in the growth step. Unlike open pond production, closed photobioreactors (PBRs) permit culture of single-species of microalgae for prolonged durations with lower risk of contamination. Hence the PBRs are appropriate for sensitive microalgae strains. Closed photobioreactor designs in a variety of configurations are currently used for microalgae cultivation. The most common PBRs are: flat-plate, tubular, and column PBRs. Researchers observed that closed PBRs have been successfully used for producing large quantities of microalgae biomass (Malinska et al., 2010). However, the costs of
closed photobioreactor are higher than that of open pond. Another challenge is the difficulty to scale-up a PBR.

1.9.3 HYBRID SYSTEMS

The hybrid system is a method that combines algae growth in a PBR and open pond. The first step is to grow algae in a PBR under controlled condition and avoiding contamination to enhance fast growth until algae reach the stationary phase, at which algae could be moved to an open pond. Transfer algae culture from photobioreactor to open pond is considered as an environmental stressing, which stimulate increasing algae lipid production with increasing the length of the growth period. However, algae culture transferred to open pond is threatened by contamination.

1.9.4 OFFSHORE MEMBRANE ENCLOSURE FOR GROWING ALGAE SYSTEM (OMEGA)

Offshore Membrane Enclosures for Growing Algae (OMEGA) is developed by NASA, (Trent, 2010 and McConnell et al. 2012). It consists of large flexible plastic containers (tubes or bags) that are at least partially permeable to CO$_2$ and O$_2$. The containers, also termed PBRs are filled with domestic waste water and float in sea water. The idea is for the photobioreactor to automatically dewater as the treated waste water leaves through forward osmosis (Christenson et al., 2011). Thus, algae are cultivated and harvested in the bags, after most of treated water is removed through liquid exchange membrane. The algae are removed and processed, and the bags are cleaned and reused
The algae clean the wastewater by removing nutrients that otherwise would contribute to marine dead zone formation.

The PBR bags can be made of plastic (e.g., polyurethane) or other impermeable materials in different sizes and shapes. The bags will have a transparent upper surface to allow light penetration and may have a reflective lower surface to increase light available for algae cultivation. The vertical spacing in the floating PBR should not exceed 10 cm to allow good light penetration. The bags are used offshore in aquatic or marine environment, which provides support, cooling, mixing, and dewatering. The bags have patches of different kinds of semi-permeable membranes: gas permeable, water permeable, and/or nutrient permeable. Patches of gas permeable membranes allow the oxygen and other gases to pass out of the bag and CO\textsubscript{2} to pass into the bag. Patches of liquid permeable membranes (forward osmosis [FO] membranes) allow the water to pass out of the bag and never allow the algae to pass through. Therefore, concentrated algae remain in the bag and are ready to be harvested. Nutrient permeable membranes can be used only if the nutrient concentration in the environment is higher than that inside the bag.

The Ocean provides a large area to grow microalgae. OMEGA benefits include cleaning wastewater, good capture of carbon dioxide and ultimately producing biofuel without competing with agriculture for water, fertilizer or land. Moreover, problems of contaminations, evaporation losses, high harvesting cost, and temperature control, caused by open pond or photobioreactors, can be avoided.
1.10 MICROALGAE LIPID TRIGGERING

Microalgae lipids are a promising feedstock for biodiesel production. These lipids can be converted into biodiesel by the transestrification process. Depending on the microalgae used, the lipid content may be as much as 20-40 g lipid/100 g dry algae. However, microalgae species divide into two categories under optimal growth conditions: (1) high growth rate and low lipid content (2) high lipid content and low growth rate. Increasing the lipids will improve the economics of biodiesel production. One possibility to increase the lipid yield in algae is to expose them to an environmental stress, often called lipid triggering, prior to microalgae harvesting and lipid extraction. Lipid triggering may involve algae nutrient deprivation e.g. nitrogen or phosphorus starvation, alternating the light exposure, chemical stressing, or thermal stressing (Elmoraghy et al., 2012). The environmental stress should cause the microalgae to “flip a switch” to turn on lipids production and turn off cell division/growth.

This study focusses on the effect of the nitrogen deprivation on the Chlorella vulgaris lipid production.
1.11 IN-SITU TRANSESTRIFICATION

Traditional microalgae biodiesel production includes two steps; lipid extraction and the transestrification of the extracted lipid. In-situ transestrification (one-step process) is a single-step integrated process. It refers to the direct transestrification of the lipids (Laurens et al., 2012), omitting the need for an initial lipid extraction and offers the advantages of quantifying algae lipids as fatty acid methyl esters (FAMEs).

1.12 PROJECT SIGNIFICANCE

There are considerable investigations in microalgae growth, production of algae oil and its use in biodiesel and bio-jet fuel production. Yet large-scale production of algae oil is still not economical. Theoretical estimates are that microalgae oil production per acre of land is almost 200-300 times other oil crops. Actual lipids are only 10-15 times other oil crops making large-scale algae production economically unattractive. Large-scale production of microalgae is energy and water intensive. Research has shown that 30% of the production cost of algae is due to growth and harvesting. Clearly, improvements in the algae growth process that would decrease the fresh water requirements, lower the energy use and make the process greener are highly desirable. For bio-jet production, additional requirements are to obtain a liquid fuel with desirable characteristics, e.g., freezing point, volume per unit energy, heat of combustion and viscosity.
1.13 HYPOTHESES

Eight hypotheses form the basis of the current project.

1. Light Emitting Diodes (LEDs) can be effectively used to grow algae with comparable or better algae lipid production using fluorescent lights, making the process greener and more energy efficient.

2. Lipid-containing microalgae species can be grown using waste water effluent from a municipal waste water treatment plant. In addition, the algae production and lipids productivity in municipal waste water will be comparable to algae growth in fresh water using the same nutrients, reducing the fresh water footprint.

3. Higher incident light intensity increases the production of algae biomass and lipids.

4. Scale-up of microalgae growth PBR will reduce algae biomass production and lipid production.

5. Physical properties of microalgae biodiesel can be predicted from the Fatty Acid composition.

6. One step process (in-situ transestrification) avoids using any hazardous materials such as hexane and saves time. Thus, in-situ transestrification improves the biodiesel production time and economics.

7. Microalgae lipid triggering by nitrogen deprivation will increase the content of algae lipid.

8. Microalgae biodiesel can be blended with petroleum based jet fuel. The resulting liquid fuel will have properties similar to jet fuel, but is a greener drop-in aviation fuel.
1.14 PROJECT GOAL

The goal of this project is to develop an economical process for sustainable microalgae bio-jet fuel production while lowering the energy and fresh water requirements. An inexpensive source of water and nutrients for the algae is municipal waste water. An inexpensive source of lighting energy for the photosynthesis of algae is light emitting diodes (LEDs). The economics of bio-jet fuel and algae oil production can be improved using the in-situ process. This research has experimented with integrating the use of waste water in microalgae growth to reduce the fresh water requirement, replacing fluorescent light with low energy LEDs to make the growth process more energy efficient, use of in-situ process and blending the resulting biodiesel with jet fuel.

1.15 PROJECT OBJECTIVES

To accomplish the above goals the specific objectives of this project are to:

1. Investigate minimizing the energy requirements for microalgae growth by replacing fluorescent lights with light emitting diodes (LEDs).

2. Investigate the use of municipal waste water in growing microalgae to reduce fresh water use.

3. Study the effect of light intensity on algae oil production.


5. Investigate the scale up microalgae growth in fresh water and waste water from 2 Liters to 80 Liters using fluorescent light and red-blue LEDs.
6. Determine the Fatty acids composition of the microalgae biodiesel and use it to predict its physical properties.

7. Study the one-step production of biodiesel using in situ algal biomass transestrification process to reduce production time and cost.

8. Study the effect of the nitrogen starvation on the fatty acid methyl esters (FAMEs) concentration.

9. Investigate biodiesel blending with jet-fuel in order to obtain bio-jet fuel.

10. Determine the properties of the blend of different biodiesel: jet-fuel ratios. The properties include specific gravity, freezing points, and heat of combustion.

1.16 PROJECT CHALLENGES AND APPROACHES

Project challenges and approaches and the corresponding objectives are summarized in Table 1.5

<table>
<thead>
<tr>
<th>CHALLENGES</th>
<th>APPROACHES</th>
<th>OBJECTIVES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduce energy requirements for microalgae growth.</td>
<td>Use low energy light emitting-diodes (LEDs)</td>
<td>1,3,4</td>
</tr>
<tr>
<td>Reduce CO₂ emissions, to make the process greener</td>
<td>Replace fluorescent light with LEDs to reduce electric power consumption and eliminate the CO₂ emission due to the eliminated electric power.</td>
<td>1,3,4</td>
</tr>
<tr>
<td>Reduce water usage for microalgae growth</td>
<td>Replace fresh water usage with waste water</td>
<td>2,3,4</td>
</tr>
<tr>
<td>Scale-up microalgae growth</td>
<td>Use 80 L cylindrical PBR with fluorescent light and LEDs for microalgae growth</td>
<td>5</td>
</tr>
<tr>
<td>Determine the biodiesel properties</td>
<td>Use the FAME composition to predicted the biodiesel properties</td>
<td>6</td>
</tr>
<tr>
<td>Reduce biodiesel production time and cost</td>
<td>Use the in-situ transestrification (one-step, or integrated extraction-transestrification) process</td>
<td>7</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>---</td>
</tr>
<tr>
<td>Increasing microalgae lipid production</td>
<td>Lipid triggering by nitrogen starvation</td>
<td>8</td>
</tr>
<tr>
<td>Bio jet-fuel production</td>
<td>Blend jet fuel with biodiesel and determine the properties of the blend</td>
<td>9,10</td>
</tr>
</tbody>
</table>

### 1.17 THESIS ORGANIZATION

The material in this thesis is organized as follows:

- Chapter 2 – Literature Review
- Chapter 3 – Materials and Methods
- Chapter 4 – Results and Discussion
- Chapter 5 – Conclusions
- Chapter 6 – Recommendations for Future Work.
CHAPTER II

2 LITERATURE REVIEW

The role of the aviation industry is the global transportation of passengers and freight, Air Transport Action Group (ATAG, 2009). Jet fuel is the common energy source to power the airplanes. Bio-jet fuels Biofuels are the alternative renewable jet fuel. Three main processes are needed until the airline can take delivery of the bio jet fuel. These are production of algae biodiesel, conversion into bio jet fuel and delivery of the product. This study focuses on the first two processes; namely algae biodiesel production and conversion to bio jet fuel.

This chapter is intended to show:

- Awareness of research in the microalgae bio-jet and biodiesel fields.
- The basis for the proposed work and its significance.
- That the proposed work fits well with what has already been done and will lead to new knowledge.

2.1 ALGAE BACKGROUND

Algae are found almost everywhere. They grow in both fresh and saline water, in cold streams and in hot swamps and ponds, at room temperature and bright sunny environment (Wilson et al., 2012). Algae are considered as little factories that grow in an aquatic environment, use light energy and carbon dioxide to create biomass. Algae size range can be as little as few micrometers to as long as 30 m.
2.1.1 ALGAE CLASSIFICATION BY SIZE AND GROUP

Algae are classified according to their size into microalgae and macroalgae. Macroalgae are large algae that can be seen without a microscope (inches and greater). They are multi cellular algae often growing in ponds. The largest macroalgae are called “seaweed”. According to Thomas 2002, giant kelp is the largest seaweed and grows to about 80 m long.

Microalgae (or microscopic algae) are small microscopic aquatic photosynthetic plants (around micrometers) that require a microscope to be seen. They are single celled that grow quickly (often they double in few hours, Metting, 1996) in water suspension (Chang, 2007). Microalgae can be classified based on their pigmentation, growth conditions, cells wall structure and flagellation. There are four main groups of algae: cyanobacteria, green algae, red algae and diatomaceae. Table 2.1 gives a brief description of each group.

Table 2.1: Classification and description of microalgae groups (Rodolfi et al., 2009, Melinda et al., 2011)

<table>
<thead>
<tr>
<th>Algal Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green algae or Blue-green algae</td>
<td>Algal cells have green chloroplast that contains chlorophyll a and b. These cells have mitochondria. Some species have flagella.</td>
</tr>
<tr>
<td>Red algae or Rhodophyceae</td>
<td>Cells have chloroplast with chlorophyll a and d, and phycobillins. Cells have double cell wall, but do not have centrioles and flagella.</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>Cells contains chloroplast with no chlorophyll</td>
</tr>
<tr>
<td>Diatomaceae</td>
<td>Class of Bacillariophyceae. Diatomaceae cells have chloroplast carrying chlorophyll a and c. They have hard wall due to the presence of silica. Most of these cells can be found in fresh or salted sea. Majority of diatom species live in cold water.</td>
</tr>
</tbody>
</table>

28
According to Demirbass, 2009, the productivity of microalgae is roughly 50 times that of switchgrass. Switch grass is the fastest growing plant in the world. Certain microalgae contain large amounts of lipids within their cells, making them feedstock candidates for biodiesel. Chlorella is a microorganism of interest in this work due its growth rate and lipid content.

2.1.2 MICROALGAE CONTENT

Algae are simple organisms that use the green pigment chlorophyll a in the photosynthesis process but they lack advanced systems like roots and leaves. Microalgae contain carbohydrates, proteins and lipids (natural oils) among other compounds. The microalgae natural oils/lipids are in the forms of triacylglycerides (TAGs) (shown in Reaction 1.1, section 1.6.1)

\[ \text{H}_2\text{C} - \text{O} - \text{C} - \text{R}_1 \\
\text{H}_2\text{C} - \text{O} - \text{C} - \text{R}_2 \\
\text{H}_2\text{C} - \text{O} - \text{C} - \text{R}_3 \]

TAGs are the desired lipids to produce biodiesel, Danielo, 2005. Microalgae containing high natural oils content and low proteins content and exhibit fast growth (doubling every 3.5 hours, Chisti, 2007) would be very suitable as a biodiesel feedstock. R₁-COOH, R₂-COOH and R₃-COOH in the TAGs are the fatty acids. These could be short or long
chains hydrocarbons. Shorter chain length fatty acids (14-20 carbon atoms) are ideal for making biodiesel.

2.1.3 MICROALGAE STRAINS

The term algae species is taken to mean populations of organisms that have a high level of genetic similarity and belonging to the same genus. For example Chlorella is a genus containing about 45% protein, 20% fat, 20% carbohydrate (Belasco, 1997). Chlorella vulgaris, Chlorella spirulina, Chlorella minutissima, Chlorella pyrenoidosa and Chlorella variabilis microalgae are species of the Chlorella genus. A strain of algae is taken to mean a genetic variant or subtype of a particular species of organism. Examples would be Chlorella vulgaris Beyerinck. It is a strain of Chlorella vulgaris that could potentially be used for wastewater treatment. Chlorella strains have been considered as promising candidates for the commercial lipid production due to their faster growth and easier cultivation than other species such as Botryococcus braunii, which has high oil content but grow slowly and hence, has low oil production (Lv et al. 2010).

2.2 MICROALGAE CULTIVATION

The growth characteristics and composition of microalgae are known to significantly depend on the cultivation conditions. There are four major types of cultivation conditions for microalgae: phototrophic, heterotrophic, mixotrophic and photoheterotrophic.
2.2.1 PHOTOTROPHIC CULTIVATION

Phototrophic cultivation occurs when the microalgae use light, such as sunlight, as the energy source, and inorganic carbon (e.g., carbon dioxide) as the carbon source to form chemical energy through photosynthesis. This is the most commonly used cultivation condition for microalgae growth. Table 2.1 shows that under phototrophic cultivation, there is a large variation in the lipid content of microalgae, ranging from 4% to 68%, depending on the type of microalgae species. The major advantage of microalgae phototrophic cultivation is the consumption of CO\textsubscript{2} as a carbon source for cell growth and oil production. Microalgae can utilize waste CO\textsubscript{2} to grow, so their cultivation should be next to a fossil-fueled power plant. CO\textsubscript{2}-rich flue gas can be bubbled directly into the algae growth solution. This bubbling accomplishes two roles; providing a source of inorganic carbon and flue gas decarbonization, i.e., helping to mitigate flue gas CO\textsubscript{2} emissions (Ben-Amotz, 2007).

2.2.2 HETEROTROPHIC CULTIVATION

In addition to phototrophic growth, some microalgae species can also use organic carbon under dark conditions, just like bacteria. This is called heterotrophic cultivation. It could avoid the problems associated with limited light that inhibit high cell density in large scale photobioreactor during phototrophic cultivation. Microalgae can use different organic sources (such as glucose, acetate, glycerol, sucrose, and lactose). Heterotrophic growth gives much higher lipid productivity, nearly 20 times higher than that obtained
under phototrophic cultivation (Table 2.2). However, the sugar-based heterotrophic system frequently suffers from problems with contamination. There is also the added cost of the carbon source, i.e. sugars.

2.2.3 MIXOTROPHIC CULTIVATION

Mixotrophic cultivation is when microalgae undergo photosynthesis and use both organic compounds and inorganic carbon (CO2) as a carbon source for growth. In addition, the CO2 released by microalgae via respiration is trapped and reused under phototrophic cultivation. Compared with phototrophic and heterotrophic cultivation, mixotrophic cultivation is rarely used in microalgae oil production. (Chen et al., 2011)

2.2.4 PHOTOHETEROTROPHIC CULTIVATION

Photoheterotrophic cultivation is when microalgae require light when using organic compounds as the carbon source. The main difference between mixotrophic and photoheterotrophic cultivation is that the latter requires light as the energy source, while mixotrophic cultivation can use organic compounds to serve this purpose. Using this approach is very rare, as is the case with mixotrophic cultivation.

Table 2.2: Lipid content and productivities of different microalgae species under different cultivation conditions (Chen et al., 2011)

<table>
<thead>
<tr>
<th>Microalgae species</th>
<th>Cultivation condition</th>
<th>Productivity of Biomass (g/l-d)</th>
<th>Lipid content (% of dcw)</th>
<th>Lipid productivity (mg/l-d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorella protothecoides</td>
<td>Heterotrophic</td>
<td>2.2-7.4</td>
<td>50.3-57.8</td>
<td>1209.6-3701.1</td>
</tr>
<tr>
<td>Chlorella sp.</td>
<td>Phototrophic</td>
<td>0.37-0.53</td>
<td>32.0-34.0</td>
<td>121.3-178.8</td>
</tr>
</tbody>
</table>
Table 2.3 shows a comparison of the characteristics of different cultivation conditions.

<table>
<thead>
<tr>
<th>Cultivation condition</th>
<th>Energy source</th>
<th>Carbon source</th>
<th>Cell density</th>
<th>Cost</th>
<th>Issues associated with scale-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phototrophic</td>
<td>Light</td>
<td>Inorganic</td>
<td>Low</td>
<td>Low</td>
<td>Low cell density</td>
</tr>
<tr>
<td>Heterotrophic</td>
<td>Organic</td>
<td>Organic</td>
<td>High</td>
<td>Medium</td>
<td>Contamination High substrate cost</td>
</tr>
<tr>
<td>Mixotrophic</td>
<td>Light and organic</td>
<td>Inorganic and organic</td>
<td>Medium</td>
<td>High</td>
<td>Contamination High equipment cost</td>
</tr>
<tr>
<td>Photo-heterotrophic</td>
<td>Light</td>
<td>Organic</td>
<td>Medium</td>
<td>High</td>
<td>Contamination High equipment cost High substrate cost</td>
</tr>
</tbody>
</table>

2.3 LIGHT SOURCE FOR MICROALGAE CULTIVATION

2.3.1 Sunlight

Microalgae have the capability to absorb light energy (photonic energy) and store it as chemical bonds, e.g. Lipids. Light source and intensity are two important parameters affecting microalgae growth system and their growth rate. Table 2.4 summarizes the advantages and disadvantages of sunlight.
Table 2.4: Advantages and disadvantages of solar energy as a light source for microalgae photosynthesis

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Sunlight is the main light source for outdoor algae growth systems.</td>
<td>- The sunlight productivity is lower than artificial lights because of daily and seasonal variations in the amount of available light.</td>
</tr>
<tr>
<td>- About 1400 W/m² of the total solar radiation reaches the earth upper</td>
<td>- Algae respiration during the night (non-light period) causes the loss of up to 25% of the algae biomass produced during the daylight (Chisti, 2007 and 2008).</td>
</tr>
<tr>
<td>atmosphere, Barsanti 2006.</td>
<td>- Over 50% of the incident sunlight has wavelengths too long to be absorbed, while some of the remainder is reflected.</td>
</tr>
<tr>
<td>- At mid-day, the sun light provides the highest light intensity, at 1100W/m².</td>
<td>- The rates of photosynthesis in bright sunlight sometimes exceed the needs of the algae, resulting in the formation of excess sugars and starch. Then the algae photosynthesis is slowed down resulting in more absorbed sunlight to go unused.</td>
</tr>
<tr>
<td>- Free Source</td>
<td></td>
</tr>
</tbody>
</table>

2.3.2 ARTIFICIAL LIGHTS

Artificial illumination is needed for indoor growth of microalgae and to overcome the difficulties in using solar energy. Fluorescent lights are the most common indoor artificial light source. Table 2.5 lists the advantages and disadvantages of fluorescent lights.

Table 2.5: Advantages and disadvantages of fluorescent lights as a light source for microalgae photosynthesis

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Better efficiency (20% of the emission is in the visible region of the</td>
<td>- The electric energy used to operate the lights may require high capital and operating costs.</td>
</tr>
<tr>
<td>spectrum) than the incandescent light bulb (5%).</td>
<td>- The incident fluorescent light has wavelengths too long to be absorbed, while some of the remainder is reflected.</td>
</tr>
<tr>
<td></td>
<td>- Decreased intensity with age and a blackening of the inside surface.</td>
</tr>
</tbody>
</table>
Therefore, different light sources should be investigated. Solar energy can be converted into another energy source used for the indoor illumination, e.g., solar-energy-excited optical fiber systems (OF-solar). Moreover, light emitting diodes (LEDs) are potential light source for improving microalgae cultivation system. LEDs light source could result in a 50% decrease in power consumption, e.g., from 40.32 to 20.16 KW-h (Table 3.3) when replacing fluorescent light at the same light intensity (Chen et al. 2011, Elmoraghy et al., 2012, Eltringham et al, 2013, Price et al. 2013). Table 2.6 summarizes the features and electricity consumption of using different light sources for microalgae growth.

Chen et al., 2011 showed that light intensity decreases exponentially with the distance from the wall of the photobioreactor as the concentration of the cells and product increase according to equation 2.1

\[
\frac{I_L}{I_0} = \exp(-YL)
\]  
Equation 2.1

where \( I_L \) is the light intensity at distance \( L \), \( L \) is the distance from the wall of the photobioreactor, \( I_0 \) is the original incident intensity and \( Y \) is the turbidity coefficient. \( Y \) increases as the concentration of the cells and product increase in the PBR.
Table 2.6: Features and electricity consumption for different artificial light sources (Chen et al., 2011)

<table>
<thead>
<tr>
<th>Light Source</th>
<th>Feature</th>
<th>Operating stability</th>
<th>Electric consumption of light source(^a) (KW-h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional artificial sources</td>
<td>Higher biomass productivity, higher stability, large illumination area, low constructing cost</td>
<td>High</td>
<td>40.32</td>
</tr>
<tr>
<td>LED</td>
<td>Lower power consumption (1 to 3 volts at 10 to 100 milliamperes), lower heat generation, longer life-expectancy, tolerate higher frequency of on-off switching, higher stability, low constructing cost, small enough to fit into virtually at any Photobioreactor compared with fluorescent lamps.</td>
<td>High</td>
<td>20.16</td>
</tr>
<tr>
<td>Optical fiber excited by solar energy (OF-solar)</td>
<td>Low electricity consumption, good light path, uniform light distribution, lower space requirement, low contamination risk, lower cost</td>
<td>Low</td>
<td>1.0</td>
</tr>
</tbody>
</table>

\(^a\) The electricity consumption of light sources was based on a 40 L photobioreactor.

2.3.3 LIGHT EMITTING DIODES

Achieving maximum light efficiency of the microalgae photosynthetic processes requires that the spectrum of the emitted light be in the Photosynthetically Active Radiation (PAR) part of the solar spectrum. Algae can use the PAR only in the photosynthesis process. PAR is about 48% of the total solar radiation. The longer wavelengths (e.g., infrared) do not have energy to initiate the photosynthesis process. Shorter wavelengths (e.g., Ultraviolet or UV) have too much energy and can damage the phototrophic growth of algae. This means that at least 52% of the solar energy is not used.
by the algae. Lights generated by the light emitting diodes (LEDs) have a narrow spectrum. For example a red LED will generate and emit lights around the wave length of the red color. Thus, all the light emitted by the red LED can be fully absorbed by the microalgae. This is the major advantage of LEDs.

Wang et al., 2007 found that the highest specific growth rate and biomass production were obtained by using red LED in the Photoautotrophic cultivation of Spirulina platensis.

Matthijs et al, 1995 used standardized panel with 2 LEDs/ cm² fully covered one side of the culture vessel to grow green algae *Chlorella pyrenoidoa*. They demonstrated that at standard voltage in continuous operation, the light output of the light emitting diodes panels appeared more than sufficient to reach optimal growth. Figure 2.1 shows the design of their culture vessel illuminated with the LEDs panel.

![Figure 2.1: Picture of the culture vessel illuminated by the LEDs panel (Matthijs et al., 1995)](image)
This discussion/review leads us to the conclusion that LEDs are the most suitable illumination sources for microalgae growth, which requires low to medium intensity levels light at a set wavelength. This work compares the use of Fluorescent and LED light sources. Eltringham et al. (2013) are studying advanced use of LEDs to grow microalgae.

2.4 MICROALGAE CULTIVATION IN WASTE WATER

The three primary nutrients required for algae growth are nitrogen, phosphorous, and carbon. The amount of nitrogen required as fertilizer/nutrient is determined to be 8-16 tons Nitrogen/ hectare (N/ha). Using this large amount of fertilizer to grow microalgae make microalgae compete with the food crops that needs fertilizer to grow. Moreover, the expense of this amount of fertilized increases the cost of algae biomass production. For instance, the cost of the fertilizer is half of the overall cost of Spirulina cultivation (Markou et al., 2011). On the other hand, using waste water rich in N and P as a nutrient medium for microalgae growth reduces the use of the fertilizer. At the same time, microalgae reduce the amounts of these organic and inorganic compounds in waste water, and provide biological method for waste water treatment. Therefore, using waste water reduces fresh water usage and decreases the cost of algae biomass production for better economic biodiesel production (Christenson et al., 2011). Research is being performed to explore microalgae growth in waste water, (Greer et al. 2009, Woertz 2009, Craggs et al. 2011, Zuka et al. 2012, Chaput et al 2012, Elmoraghy et al. 2012 and Price et al. 2013). Some studies looked at offshore algae growth in wastewater (McConnell et al. 2012). The U.S. Department of Energy has recognized the potential synergy of wastewater treatment and biofuel production from algae, stating, “Inevitably, wastewater treatment
and recycling must be incorporated with algae biofuel production (U.S. DOE, 2010).”

Because much of the infrastructure is already in place, algae based wastewater treatment can be deployed relatively soon. The use of wastewater can offset the cost of commercial fertilizers otherwise needed for the production of algae, and wastewater treatment revenues can offset algae production costs. It is apparent that overcoming the current challenges to the production and harvesting of algae will be beneficial for both wastewater treatment and for the production of biofuels and bioproducts.

According to the 2008 Clean Watersheds Needs Survey, the total of reported wastewater flow in the U.S. is 32,345 million gallons per day (122,439 million liters per day) (U.S. EPA, 2008). Using medium strength domestic wastewater values, there is enough N (40 mg/l or 40 ppm) and P (8 mg/l) in each liter to produce 0.6 g of algae per liter (Table 2.7) for a total of 77.6 million kg of algae/day. Assuming 90% removal of the limiting nutrient a 10% (mass of biodiesel/mass of algae) biodiesel, a biodiesel density of 0.801 kg/l, and 9 months per year operation, an average biodiesel production of roughly 1.7 million gallons/day is calculated (6.5 million liters/day). Although this is only a small fraction of the 378 million gallons of transportation fuel the U.S. uses per day (U.S. EIA, 2009), large-scale biodiesel production is not favorable without waste water treatment as a primary goal.
Mostafa et al., 2012 demonstrated that Microalgae cultivation in waste water media is suitable and non-expensive method when compared with conventional cultivation growth for sustainable biodiesel production. They built their research on growing nine species of algae in different waste water treatments namely: without treatment, after sterilization, with nutrients with sterilization and with nutrients. Their results showed that the highest biodiesel production from algal biomass cultivated in waste water was obtained by Nt stomoc humifulum (11.8 %) when cultivated in waste water without treatment and the lowest (3.8 %) was recorded by Oscillatoria sp. when cultivated on the sterilized-domestic waste water.
Govindarajan et al., 2010 proved that microalgae have the ability to grow in an unaided environment as well as take up the nutrient available in the growth medium such as domestic, industrial waste water. They use the desalination reject stream that is available from the desalination plants in Oman as the growth medium to grow different species of microalgae. They were able to obtain 4.8 g dry algae/50 ml of medium through 24 days of growth period.

There are disadvantages to the use of waste water for microalgae growth. These include:
- Difficulty in settling algae upon harvesting,
- Waste water effluent exceeds the suspended solids limits (about 45 mg/l).
- Algae use interferes with disinfection
- Biomass produced, if not totally settled and removed
- Use of chemicals, e.g., aluminum sulfate, to settle the algae add to the biodiesel cost.

This discussion/review leads us to the conclusion that waste water is promising for microalgae growth and economic biodiesel production. This work compares the use of waste water versus fresh water (reverse osmosis water) to grow microalgae and produce natural lipids/oil.

2.5 PHOTOBIOREACTORS FOR MICROALGAE CULTIVATION

There are several advantages and disadvantages of closed photobioreactors (Malinska et al., 2010, Ferrentino 2007, Mulumba 2010). These are given in Table 2.8
Table 2.8: Advantages and disadvantages of closed photobioreactors

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Controlled cultivation of algae, hence potential for much higher productivity</td>
<td>- Capital cost is very high. This is the most important bottleneck that is hindering the progress of algae fuel industry.</td>
</tr>
<tr>
<td>- Large surface-to-volume ratio. PBRs offer maximum efficiency in using light and therefore greatly improve productivity.</td>
<td>- Despite higher biomass concentration and better control, data accumulated in the last two decades have shown that the productivity and production cost in some enclosed photobioreactor systems are not much better than those achievable in open-pond cultures.</td>
</tr>
<tr>
<td>- The culture density of algae produced is about 30 times greater than open ponds.</td>
<td>- Technical difficulty in sterilizing has hindered their application for algae culture for specific products such as high value pharmaceutical products.</td>
</tr>
<tr>
<td>- Better environmental control of important parameters, e.g., temperature, pH, etc.,</td>
<td></td>
</tr>
<tr>
<td>- Better control of gas transfer.</td>
<td></td>
</tr>
<tr>
<td>- Reduction in evaporation of growth medium.</td>
<td></td>
</tr>
<tr>
<td>- More uniform temperature.</td>
<td></td>
</tr>
<tr>
<td>- Better protection from outside contamination.</td>
<td></td>
</tr>
<tr>
<td>- Space saving - can be mounted vertically, horizontally or at an angle, indoors or outdoors.</td>
<td></td>
</tr>
<tr>
<td>- Reduced Fouling – Some PBRs include a self-cleaning mechanism, which can dramatically reduce fouling.</td>
<td></td>
</tr>
</tbody>
</table>

Photobioreactors are classified according to their shapes into different types: flat plate, tubular, and column. Each has advantages and disadvantages.

2.5.1 FLAT PLAT PHOTOBIOREACTORS

Flat plate PBR is made from transparent material for maximum light energy capture, and a thin layer is required to allow light penetration. Flat plat PBR enhances large illumination surface area and accordingly high photosynthetic efficiency is achieved when compared to tubular PBR. Moreover, flat plate PBR offers low accumulation of dissolved oxygen. Therefore, high algae biomass productivity can be obtained.
2.5.2 TUBULAR PHOTOBIOREACTOR

Tubular photobioreactor consists of an array of straight glass or plastic tubes. This tubular array captures the light and can be aligned horizontally, vertically, inclined, and as a helix. The tubes are generally 0.1 m or less in diameter. This is important to ensure good light penetration (Mulumba, 2010 and 2012). Figure 2.3 shows a horizontal tubular photobioreactor consists of two main parts: airlift system and solar receiver. The airlift system allows for the transfer of O₂ out of the system and transfer of CO₂ into the system as well as providing a mean to harvest the biomass. It also provides a mixing mechanism, which is very important for the system to enhance gas exchange in the tubes. The solar receiver provides a platform for the algae to grow by giving a high surface area to volume ratio. Tubular PBR deemed to be suitable for outdoor biomass cultures since they expose large surface area to sunlight (Brennan et al., 2010).
2.5.3 COLUMN PHOTOBIOREACTOR

Column PBRs offer the most efficient mixing, the highest volumetric mass transfer rates and the best-controlled growth conditions. These advantages result in high growth rate. They are very low cost and easy to operate. They can be illuminated through transparent wall or internally. The vertical column PBRs are aerated from the bottom. Column PBR offer the lowest space compared to open pond or the other types of PBRs. Some other advantages and limitations of open ponds and different types of photobioreactors are summarized in Table 2.9
Table 2.9: Advantages and limitations of open ponds and photobioreactors
(Brennan et al, 2010)

<table>
<thead>
<tr>
<th>Production system</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raceway pond</td>
<td>Relatively cheap</td>
<td>Poor biomass productivity</td>
</tr>
<tr>
<td></td>
<td>Easy to clean</td>
<td>Large area of land required</td>
</tr>
<tr>
<td></td>
<td>Utilizes non-agricultural land</td>
<td>Limited to a few strains of algae</td>
</tr>
<tr>
<td></td>
<td>Low energy input</td>
<td>Poor mixing, light, CO₂ utilization</td>
</tr>
<tr>
<td></td>
<td>Easy maintenance</td>
<td>Cultures are easily contaminated</td>
</tr>
<tr>
<td>Tubular photobioreactor</td>
<td>Large illumination surface area</td>
<td>Some degree of wall growth</td>
</tr>
<tr>
<td></td>
<td>Suitable for outdoor cultures</td>
<td>Fouling</td>
</tr>
<tr>
<td></td>
<td>Relatively cheap</td>
<td>Required large land space</td>
</tr>
<tr>
<td></td>
<td>Good biomass productivities</td>
<td>Gradients of PH, dissolved oxygen and CO₂ along the tubes</td>
</tr>
<tr>
<td>Flat plate photobioreactor</td>
<td>High biomass productivities</td>
<td>Difficult scale-up</td>
</tr>
<tr>
<td></td>
<td>Easy to sterilize</td>
<td>Difficult temperature control</td>
</tr>
<tr>
<td></td>
<td>Low oxygen build-up</td>
<td>Small degree of hydrodynamic stress</td>
</tr>
<tr>
<td></td>
<td>Readily tempered</td>
<td>Some degree of wall growth</td>
</tr>
<tr>
<td></td>
<td>Good light path</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Large illumination surface area</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Suitable for outdoor cultures</td>
<td></td>
</tr>
<tr>
<td>Column photobioreactor</td>
<td>Compact</td>
<td>Small illumination area</td>
</tr>
<tr>
<td></td>
<td>High mass transfer</td>
<td>Expensive compared to open ponds</td>
</tr>
<tr>
<td></td>
<td>Low energy consumption</td>
<td>Shear stress</td>
</tr>
<tr>
<td></td>
<td>Good mixing with low shear stress</td>
<td>Sophisticated construction</td>
</tr>
<tr>
<td></td>
<td>Easy to sterilize</td>
<td></td>
</tr>
</tbody>
</table>

This discussion/review leads us to the conclusion that photobioreactors (PBRs) are the most efficient algae growth cultivation system but more investigations need to be done in order to increase algae biomass production. This study uses two types of PBRs, 2L PBR and 80L Column PBR.
2.6 KINETICS MODEL OF MICROALGAE GROWTH

Microalgae oil has the potential to be a viable feedstock for biodiesel and renewable green aviation fuel (bio-jet fuel). This research is to demonstrate that microalgae are capable of producing bio-jet fuel similar to Jet A aviation fuel. The economics are still not favorable.

Broere (2008) estimated that closed microalgae photobioreactor systems will only be able to compete with crude oil when the price reaches US$800 per barrel (US$18 per gallon). Kanellos (2009) indicated that Solix Biofuels has developed technologies to produce oil derived from algae at about $33 per gallon. The current cost of crude oil is about $100 per barrel or about $2.25 per gallon. Reducing the cost of microalgae oil motivates the development of kinetic models that can describe microalgae growth and predict the algae growth rate. Thus, Kinetic studies are so important for designing microalgae photobioreactors.

Pai et al., 2011 studied the kinetics of batch microalgae cultivation system. Algae were grown in circular chambers with volume of 1 liter. A magnetic stirrer was providing for stirring. Light intensity of 2300 LUX and temperature of 30 °C were controlled. Soxhlet lipid extraction method with hexane as a solvent was used to determine the oil content in algae biomass. Two first order equations were employed to analyze the growth kinetic of algae and oil content as follows.

\[
\frac{dC_{algae}}{dt} = k_{algae} C_{algae} \quad \text{Equation 2.2}
\]

\[
\frac{dC_{oil}}{dt} = k_{oil} C_{oil} \quad \text{Equation 2.3}
\]
where $C_{algae}$ is the algae concentration (mg algae biomass/L of solution) at time $t$ (day), $k_{algae}$ is the growth rate constant of algae (1/day), $C_{oil}$ is the oil concentration at time $t$ (mg/l of solution) and $k_{oil}$ is the production rate constant of algae oil (1/day).

The experimental data of algae concentration and oil content were fitted to these two first-order equations by non-linear regression method. First order equation was found to be the excellent fit model for describing the growth of algae biomass and production oil content as shown in figure 2.4 and figure 2.5.

![Figure 2.4: Curve fitting of algae biomass grown in circular chambers with volume of 1 liter. A magnetic stirrer was providing for stirring. Light intensity of 2300 LUX and temperature of 30 °C were controlled (Pai et al., 2011)
Figure 2.5: Curve fitting of algae oil content, mg/l. Algae biomass grown in circular chambers with volume of 1 liter. A magnetic stirrer was providing for stirring. Light intensity of 2300 LUX and temperature of 30 °C were controlled. Soxhlet extraction method was used to determine the algae oil content (Pai et al., 2011).

The R-squared values ($R^2$) in the first order equations for describing the growth of algae biomass production and oil content were 0.9679 and 0.973 respectively. These two equations were described as follows:

$$C_{algae} = C_{algae,0}e^{0.3095t} \quad \text{Equation 2.4}$$

$$C_{oil} = C_{oil,0}e^{0.3738t} \quad \text{Equation 2.5}$$

Where $C_{algae,0}$ and $C_{oil,0}$ is the algae concentration and oil weight in algae solution at initial time, respectively (mg/l). Equations 2.4 and 2.5 showed that the values of $k_{oil}$ was about 1.2 times as that of the values of $k_{algae}$, indicating unbalance growth of algae biomass and oil content. Therefore, cultivation conditions should be investigated to obtain higher oil content.
Huesemann, 2009 developed a kinetic model for the determination of the Maximum microalgae specific growth rate ($\mu_{\text{max}}$) in batch culture. The maximum specific growth rate is only determined during the exponential growth period and was calculated by the following equation.

$$\mu_{\text{max}} = \frac{1}{\Delta t} \ln \left( \frac{C_f}{C_i} \right)$$

Equation 2.6

Where $C_i$ and $C_f$ are the initial and final microalgae biomass concentration in mg/l, respectively, and $\Delta t$ is the length of the incubation period in days.

Boija (2008) developed the following kinetic equation for batch culture growth of Chlorella zofingiensis.

$$\ln \left( \frac{X}{X_0} \right) = \mu t$$

Equation 2.7

where $X_0$ is the concentration of chlorophyll a or total chlorophyll (mg/l) at the beginning of the exponential phase, $X$ is the microalgae concentration at time $t$, $\mu$ is the specific growth rate (day$^{-1}$), and $t$ is the operating time (day). He considered the measurements of chlorophyll a and total chlorophyll concentrations as indirect indicators of the microalgae concentration, taking into account that these parameters are directly proportional to the microalgae concentrations. They demonstrated that the plot of the logarithm ($\ln \left( \frac{X}{X_0} \right)$) versus the time in the exponential growth phase must result in a straight line with intercept at the origin equal to zero and slope with a value equivalent to the specific growth rate. The kinetics model used by Boija is similar to Huesemann model.

Algae growth kinetics can be described by many equations such as Michaelis-Menten or Monod equations and Haldane equation. Monod equation can be used as light limited growth. Kinetic model with $\mu_{\text{max}}$ is the maximum specific growth rate (h$^{-1}$), $K_l$ is the
saturation constant ($\mu$mol.m$^{-1}$.s$^{-1}$) for light intensity and $I$ is the light intensity ($\mu$mol.m$^{-1}$.s$^{-1}$). Haldane model can be used in case of light limited growth as well. Equations 2.8 and 2.9 express Monod and Haldane models respectively.

\[
\mu = \mu_{max} \frac{I}{I + K_1} \quad \text{Equation 2.8}
\]

\[
\mu = \mu_{max} \frac{I}{I + K_1 + \frac{I}{K_2}} \quad \text{Equation 2.9}
\]

Where $K_1$ ($\mu$mol.m$^{-1}$.s$^{-1}$) indicates how fast the optimum for the specific growth rate $\mu_{max}$ (h$^{-1}$) is reached. The smaller $K_1$ the faster $\mu_{max}$ is reached. $K_2$ ($\mu$mol.m$^{-1}$.s$^{-1}$) is the inhibition parameter. The smaller $K_2$ the larger the inhibition effect of the light intensity.

Figures 2.6 (a) and (b) show specific growth rate (h$^{-1}$) related to the light intensity ($\mu$mol/m$^2$s) according to Monod and Haldane equations respectively.

![Figure 2.6](image)

**Figure 2.6:** Specific growth rate, $\mu_{max}$ (h$^{-1}$) as a function of light intensity, $I$ (\mu$mol/m^2$s) for Monod model, graph (a) and Haldane model, graph (b) (Hermanto, 2009). Dunaliella tertiolecta were cultivated using three light sources; Red LEDs (2200 \mu$mol/m^2$s), Red-Blue LEDs (2800 \mu$mol/m^2$s) and 60 tungsten-halogen lamps (1800 \mu$mol/m^2$s) with initial algae concentration 1 g/l
The present study will focus on developing a kinetics model of microalgae biomass growth in a batch or semi-batch photobioreactor. McConnell (2013) has studied the kinetics of microalgae oil extraction using hexane solvent.

This discussion/review leads us to the conclusion that different kinetics model can be developed for microalgae growth and the specific growth rate should be determined for designing an efficient PBR.

2.7 LIPID TRIGERRING

Some microalgae have high oil content. These can be induced to produce higher concentration of lipids. Different triggering techniques have been searched, e.g., low nitrogen media, high Fe\(^{3+}\) concentration, salinity induced, pH change, UV Irradiance, and temperature stress).

Nitrate compound is one of the most important nutrients for algae growth. The reduction of nitrate concentration limits protein biosynthesis thus increasing the lipid/protein ratio (Converti et al., 2009). Gouveia et al., 2009 demonstrated that Neochloris oleabundans (fresh water microalgae) and Nannochloropsis sp. (marine microalgae) are suitable raw materials for biofuels production, due to their high oil content (29.0 and 28.7\% respectively). Both microalgae, when cultivated under nitrogen shortage, after 5 days of nitrogen starvation, showed a great fatty acid content increase of \(~50\%\) with no significant change in fatty acid profile.
Converti et al., 2009 studied the effect of the nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris. The concentration of nitrate (g/l) in both media for Nannochloropsis oculata and Chlorella vulgaris batch growth was reduced to half and quarter of the standard media. Their results of Chlorella vulgaris showed that the reduction of NaNO₃ concentration (from 1.5 to 0.375) resulted in threefold increase in the lipid content (from 5.9 to 15.31, g lipid/100 g dry algae) while its specific growth rate (day⁻¹) was not significantly affected. In contrast, Nannochloropsis oculata showed a gradual decrease in the growth rate accompanied by almost a duplication of the lipid content. Tables 2.10 and 2.11 show their results for Chlorella vulgaris and Nannochloropsis oculata respectively. Based on their results, they concluded that the most effective biodiesel production could be obtained when the optimum compromise of the slow growth rate and the high lipid content could be reached. This resulted in higher lipid production (See figure 2.7 (a) and (b) for Chlorella vulgaris lipid content and lipid production respectively and figure 2.8 (a) and (b) for Nannochloropsis oculata lipid content and lipid production respectively).

Table 2.10: Lipid production of Chlorella vulgaris at different nano₃ concentrations in the growth medium (Converti et al., 2009)

<table>
<thead>
<tr>
<th>NaNO₃ (g/l)</th>
<th>NaNO₃ (mM)</th>
<th>μ(day⁻¹)</th>
<th>g lipid/100 g dry algae</th>
<th>mg lipids/L-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.500</td>
<td>17.65</td>
<td>0.14±0.00</td>
<td>5.90±0.42</td>
<td>8.16±0.65</td>
</tr>
<tr>
<td>0.750</td>
<td>6.62</td>
<td>0.14±0.01</td>
<td>14.37±0.64</td>
<td>20.44±0.75</td>
</tr>
<tr>
<td>0.375</td>
<td>4.41</td>
<td>0.13±0.00</td>
<td>15.31±0.51</td>
<td>20.30±0.40</td>
</tr>
</tbody>
</table>
Figure 2.7: Effect of sodium nitrate concentration on Chlorella vulgaris lipid content, graph (a) and lipid production, graph (b).
Plot is based on the data of Converti et al., 2009.

Table 2.11: Lipid production of nannochloropsis oculata at different nano₃ concentrations in the growth medium (Converti et al., 2009)

<table>
<thead>
<tr>
<th>NaNO₃ (g/l)</th>
<th>NaNO₃ (mM)</th>
<th>μ (day⁻¹)</th>
<th>g lipid/100 g dry algae</th>
<th>mg lipids/L-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.30</td>
<td>3.53</td>
<td>0.13±0.00</td>
<td>7.88±0.21</td>
<td>10.01±0.16</td>
</tr>
<tr>
<td>0.15</td>
<td>1.76</td>
<td>0.10±0.00</td>
<td>13.01±0.39</td>
<td>13.61±1.10</td>
</tr>
<tr>
<td>0.075</td>
<td>0.88</td>
<td>0.10±0.00</td>
<td>15.86±0.59</td>
<td>16.41±0.11</td>
</tr>
</tbody>
</table>
Figure 2.8: Effect of sodium nitrate concentration on Nannochloropsis oculata lipid content, graph (a) and lipid production, graph (b). Plot is based on the data of Converti et al., 2009.

Lv et al., 2010 has done another study that focused on enhancing the lipid production of Chlorella vulgaris. It demonstrated that low nitrate concentration (0.2-3.0 mM = 20.2-303 mg potassium nitrate (MW = 101.13) /L of algae medium) limited the cell growth, and the increase of nitrate concentration would improve the growth of Chlorella vulgaris. The effect of KNO₃ concentration on the lipid production showed that 22.5%, 20.0%, 18.5%, and 15.9% were obtained at KNO₃ concentrations of 0.2, 1.0, 3.0, and 5.0 mM = 20.2, 101, 303, and 505 mg potassium nitrate/L of algae medium), respectively. These results, shown in Figure 2.9 indicated that the lipid content increased with the decrease of the KNO₃ concentration.
Although the highest biomass production obtained at the KNO$_3$ of 5.0 mM, the maximum lipid production of 40 mg/l-day was obtained at 1.0 mM. Because the highest biomass production was compromised by the lowest lipid content, a relatively lower lipid production of 35 mg/l-day was obtained at 5 mM (Lv et al., 2010).

Mallick et al., 2012 studied the effects of nitrate, phosphate, and iron limitation on Chlorella vulgaris lipid accumulation, Chlorella vulgaris cells were grown under different concentrations of nitrate (0.005 - 0.1 g L$^{-1}$, or 5 to 100 ppm) and phosphate (0.005 - 0.1 g L$^{-1}$). N-deficiency was achieved by substituting KNO$_3$ of the medium with equimolar concentrations of KCl. For P-deficiency, cultures were transferred to mineral salt, in which Na$_2$HPO$_4$·H$_2$O and KH$_2$PO$_4$ were replaced by equimolar concentrations of Na$_2$SO$_4$ and KCl, respectively. To study the effect of iron limitation, Chlorella vulgaris culture was grown at different concentrations of iron (0.0015- 0.006 g L$^{-1}$). Fe-deficiency was achieved by substituting FeSO$_4$·H$_2$O in the medium with equimolar concentrations of Na$_2$SO$_4$. Profound increase in the lipids was observed under N and P deficiency as they
reached 42.4 and 40.8% dry cell weight (dcw) respectively, on day 7 of N and P deficiency, and 38.9% (dcw) when iron concentration was reduced to 3 mg L\(^{-1}\) from the initial value of 6 mg L\(^{-1}\) on day 12 of incubation. Table 2.12 shows the optimum condition of the critical variables for maximum lipid accumulation for their study.

Table 2.12: Optimum condition of the critical variable for maximum lipid accumulation (Mallick et al., 2012)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Optimum Concentration mg/l</th>
<th>Lipid accumulation (% dcw)</th>
<th>Predicted</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>25</td>
<td>57.6</td>
<td>55.3±1.03</td>
<td></td>
</tr>
<tr>
<td>Phosphate</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubation Period (days)</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Moreover, Aquatic Energy company uses an additional production stage after sufficient growth has been achieved in clay lined raceway. After the raceway, cells enter a secondary stress pond for nitrogen starvation and lipid accumulation for 48 h before being harvested (Christenson et al., 2011).

2.8 ALGAE HARVESTING TECHNIQUES

According to Chen et al., 2011, the high cost of algae harvesting can be attributed to the low mass fractions in the culture medium (from 0.1 to 2 g dried microalgal biomass per L culture, depending on cultivation medium). Therefore, efficient harvesting methods are developed. These include using micro screens, centrifugation, flocculation, gravity sedimentation, and broth filtration (Elmoraghy et al., 2012, Chen et al., 2011, Webster
Such methods used to dewater the microalgae culture to a concentration between 10 to 450 g dried microalgal biomass/L of culture. When dewatered beyond 200 g dried microalgal biomass/L culture, the concentrate is transformed to a sludge suspension and is often referred to as paste or pellet (Halim et al., 2012). The selection of harvesting technique is dependent on the properties of microalgae such as density, size and value of the desired products (Brennan et al., 2010). High speed centrifugation (5,000 rpm for 15 min) was used in the present work to avoid the addition of flocculants, and for the ease of separation the solid from the liquid solution. Centrifugation is also suitable for most microalgae. After centrifugation, the algae cells are freeze dried for approximately 48 h, producing dry algae flakes that can then be crushed into a powder using a mortar and pestle. Then lipids should be extracted from algae powder.

2.9 ALGAE LIPID EXTRACTION

Dry algae biomass powder is mixed with hexane, the extraction solvent. Once the crude lipids are separated from the crushed microalgae cell powder, the extraction solvent, and water, their mass can be measured gravimetrically. Lipid extraction technology for microalgae biodiesel production needs are summarized in Table 2.13

Table 2.13: Requirements of microalgae lipids extraction technology

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Explanation/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-lipid Contaminant</td>
<td>Minimize the co-extraction of non-lipid contaminations, such as proteins and carbohydrates.</td>
</tr>
<tr>
<td>Selectivity</td>
<td>Higher selectivity towards acylglycerols than other lipid fractions that are not as readily convertible to biodiesel, i.e., free fatty acids, hydrocarbons, and chlorophylls.</td>
</tr>
<tr>
<td>Time and Energy</td>
<td>Time and energy Efficient</td>
</tr>
<tr>
<td>Costs</td>
<td>relatively inexpensive in terms of operating and capital costs</td>
</tr>
<tr>
<td>Safety</td>
<td>Low or no hazardous solvent use</td>
</tr>
</tbody>
</table>

57
2.10 CONVENTIONAL TRANSESTRIFICATION

Triacylglycerides (TAGs) extracted from dry algae can be easily converted into biodiesel through the transestrification reaction. These lipids have a common structure of triple esters where usually three long chain fatty acids (FAs) are coupled to a glycerol molecule. Transestrification displaces glycerol with small alcohols (e.g., methanol) to produce fatty acid methyl ester (FAME), according to Reaction (1.1).

Plant oils and animal fats themselves have unfavorable characteristics that do not allow them to compete with biodiesel. Therefore, they are transestrified into methyl esters of fatty acids known as biodiesel FAME (Wcislo et al., 2008).

Rajan et al., 2010 demonstrated that the properties of Jatropha oil methyl esters are very similar to those of diesel. The transestrification improved the important fuel properties like specific gravity, viscosity and flash point. Table 2.14 lists the properties of diesel, Jatropha oil and its methyl ester (JOME).

Table 2.14: Properties of diesel, Jatropha oil and Jatropha oil methyl ester (JOME) (Rajan et al., 2010).

<table>
<thead>
<tr>
<th>Property</th>
<th>Diesel</th>
<th>Jatropha Oil</th>
<th>JOME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (kg/m³)</td>
<td>840</td>
<td>918</td>
<td>880</td>
</tr>
<tr>
<td>Viscosity at 40°C (mm²/s)</td>
<td>3.8</td>
<td>38.23</td>
<td>4.18</td>
</tr>
<tr>
<td>Calorific value C.V., or Heating Value (MJ/kg)</td>
<td>43.5</td>
<td>39.77</td>
<td>38.45</td>
</tr>
<tr>
<td>Flash point (°C)</td>
<td>52</td>
<td>261</td>
<td>248</td>
</tr>
<tr>
<td>Fire point (°C)</td>
<td>63</td>
<td>302</td>
<td>292</td>
</tr>
<tr>
<td>Cetane number</td>
<td>47</td>
<td>40-45</td>
<td>52</td>
</tr>
</tbody>
</table>
Transestrification reaction is carried out using catalyst to accelerate the reaction. The catalyst can be acid, alkali or an enzyme. The advantages and challenges of each catalyst are summarized in Table 2.15

<table>
<thead>
<tr>
<th>Catalysts</th>
<th>Advantage/Use</th>
<th>Challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td>Useful for the conversion of high free fatty acids (FFAs) feedstocks to fatty acids esters (biodiesel)</td>
<td>Very low reaction rate converting triglycerides to FAMEs. (Gerpen, 2005), (Mousdale, 2008)</td>
</tr>
<tr>
<td>Alkali or Alkaline (most frequently used)</td>
<td>Higher reaction rates (4000 times faster) than acid catalyst</td>
<td>FFAs may react with alkali to form soap and water. More alkali is needed. FFA + KOH $\rightarrow$ K-Soap + water When FFA is greater than 5%. The resulting soap will emulsify FAMEs and glycerol.</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Good tolerance to FFAs in the feedstocks</td>
<td>Expensive, may not be able to provide high quality biodiesel to meet ASTM specification</td>
</tr>
</tbody>
</table>

Alkali catalysts have faster reaction rate (estimated at 4000 times faster) and higher conversions than acid catalysts (Halim et al., 2012). However, it also depends on the type of the lipids that are transestrified. However, acid catalyst has the ability to catalyze the esterification of all free and linked fatty acids. It requires even heating and longer time compared to base catalyst (Laurens et al., 2012).

### 2.11 IN-SITU TRANSESTRIFICATION

Several recent studies, e.g. Ferrentino (2007), Mulumba (2010, 2012) investigated simultaneous microalgae oil extraction and transestrification process to produce biodiesel from dry microalgae. This process, also termed direct transestrification or in-situ
transestrification since it combines lipid extraction and transestrification in a single step. In this process, oil-bearing dried algae are sonicated to crack the algae outer shell. Then reacted directly with the alcohol and catalyst, thereby eliminating the need for pre-extracted oil, and its associated capital and intensive running cost production methods. Various parameters affect the conversion, reaction rate and quality of the biodiesel in in situ transestrification. These include: agitation intensity, molar ratio of alcohol to oil, reaction temperature, and alcohol type. Advantages of the in-situ process include: one step for extraction and transestrification of lipids to biodiesel; No need for hazardous chemical solvents, like hexane, reduces processing time; Production and recovery of FAME can be done within 90 minutes with very good recovery of algae oils Georgogiannia et al. 2008, and Qian et al., 2008. Among the disadvantages, the reaction (sonication) time has significant effect on the FAME content. The sonication time effect is twofold in the extraction and transestrification. The sonication increases the temperature and hence improves the methanol extraction of microalgae oil. Higher sonication times (above 10 minutes, Ferrentino, 2007) are inefficient as they may result in overheating of the reaction mixture and more losses of the methanol and the biodiesel. In addition, the scale up of the in-situ may be very challenging (Patil et al. 2010).

2.12 MICROALGAE LIPID FATTY ACIDS

Microalgae have both polar and non-polar (neutral) lipids. The neutral lipids are the oils used to create biodiesel. Triglycerides-esters are the major components of oils. These consist of one molecule of glycerol and three molecules of fatty acids. The characteristics of oil are usually described in terms of its fatty acids composition. Fatty acids have a
carboxyl group, which is polar and a hydrocarbon chain, which is non-polar. Fatty acids are described by the number of carbons and double bonds in the hydrocarbon chain. If there is one double bond in the hydrocarbon chain, the fatty acid (FA) is termed monounsaturated. If there are two or more double bonds it is termed polyunsaturated. If there are no double bonds in the hydrocarbon chain, the FA is considered saturated. The types of fatty acids produced by the microalgae depend on the conditions in which the cell was grown. Some of the factors, which affect lipid production include nutrient composition and availability, temperature, light intensity, and aeration rate. Usually, the microalgae fatty acids (FAs) have hydrocarbon chains ranging from 12 to 22 carbons. The extent of unsaturation can vary, but the number of double bonds never exceeds six. The unsaturated fatty acids are cis isomers. The length of the hydrocarbon chain and the degree of unsaturation influence the heating value, viscosity, cloud point, and pour point of the biodiesel, which is created (Halim et al. 2012, McConnell, 2013). Some of these effects are summarized in Table 2.16

Table 2.16: Advantages and disadvantages of saturated and unsaturated fatty acids in microalgae when converted to biodiesel

<table>
<thead>
<tr>
<th>Fatty Acid Type</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increasing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturated</td>
<td>Decreases NOx emissions</td>
<td>Increases melting point</td>
</tr>
<tr>
<td></td>
<td>Improves oxidative stability and long term storage</td>
<td>Increases viscosity</td>
</tr>
<tr>
<td></td>
<td>Reduces deposition</td>
<td>Reduces lubricity</td>
</tr>
<tr>
<td>Increasing</td>
<td>Decreases melting point</td>
<td>Increases NOx emissions</td>
</tr>
<tr>
<td>Unsaturated</td>
<td>Decreases viscosity</td>
<td>Lowers oxidative stability and long term storage</td>
</tr>
<tr>
<td></td>
<td>Improves lubricity</td>
<td>Increases deposition</td>
</tr>
</tbody>
</table>
Table 2.17 (reproduced from Mulumba 2010) lists some saturated and unsaturated fatty acids (FAs) found in microalgae cells. The name of each fatty acid (FA) is followed by the total number of carbon atoms (including the C in COOH), and total number of double bonds; for instance, (16:1) indicates the fatty acid (palmitoleic or sapienic) has 16 carbon atoms with one double bond.

Table 2.17: Saturated and unsaturated fatty acids (FAs) found in microalgae cells (Matsumoto et al., 2009; Singh and Singh, 2009)

<table>
<thead>
<tr>
<th>Category</th>
<th>FA Name</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>Capric (10:0)</td>
<td>CH₃-(CH₂)₈-COOH</td>
</tr>
<tr>
<td>Saturated</td>
<td>Lauric (12:0)</td>
<td>CH₃-(CH₂)₁₀-COOH</td>
</tr>
<tr>
<td>Saturated</td>
<td>Myristic (14:0)</td>
<td>CH₃-(CH₂)₁₂-COOH</td>
</tr>
<tr>
<td>unsaturated</td>
<td>Myristoleic (14:1)</td>
<td>CH₃(CH₂)₃CH=CH(CH₂)₂COOH</td>
</tr>
<tr>
<td>Saturated</td>
<td>Palmitic (16:0)</td>
<td>CH₃-(CH₂)₁₄-COOH</td>
</tr>
<tr>
<td>unsaturated</td>
<td>Palmitoleic (16:1)</td>
<td>CH₃(CH₂)₅CH=CH(CH₂)₂COOH (= bond at C7)</td>
</tr>
<tr>
<td>unsaturated</td>
<td>Sapienic (16:1)</td>
<td>CH₃(CH₂)₇CH=CH(CH₂)₄COOH (= bond at C10)</td>
</tr>
<tr>
<td>unsaturated</td>
<td>Hexadecadienoic (16:2)</td>
<td>CH₃(CH₂)₁₀CH=CHCH=CHCOOH</td>
</tr>
<tr>
<td>unsaturated</td>
<td>Hexadecatrienoic (16:3)</td>
<td>CH₃(CH₂)₄CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₂COOH</td>
</tr>
<tr>
<td>Saturated</td>
<td>Stearic (18:0)</td>
<td>CH₃-(CH₂)₁₆-COOH</td>
</tr>
<tr>
<td>unsaturated</td>
<td>Oleic (18:1)</td>
<td>CH₃(CH₂)₇CH=CH(CH₂)₄COOH</td>
</tr>
<tr>
<td>unsaturated</td>
<td>Linoleic (18:2)</td>
<td>CH₃(CH₂)₈CH=CHCH₂CH=CH(CH₂)₇COOH</td>
</tr>
<tr>
<td>unsaturated</td>
<td>α-Linolenic (18:3)</td>
<td>CH₃CH₂CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₇-COOH</td>
</tr>
<tr>
<td>unsaturated</td>
<td>Octadecontetraenoic (18:4)</td>
<td>CH₃CH₂CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₄-COOH</td>
</tr>
<tr>
<td>Saturated</td>
<td>Arachidic (20:0)</td>
<td>CH₃-(CH₂)₁₈-COOH</td>
</tr>
<tr>
<td>unsaturated</td>
<td>Arachidonic (20:4)</td>
<td>CH₃(CH₂)₄CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₃COOH</td>
</tr>
<tr>
<td>Saturated</td>
<td>Behenic (22:0)</td>
<td>CH₃-(CH₂)₂₀-COOH</td>
</tr>
<tr>
<td>Saturated</td>
<td>Eicosapentaenoic (20:5)</td>
<td>CH₃CH₂CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₃COOH</td>
</tr>
<tr>
<td>unsaturated</td>
<td>Erucic (22:1)</td>
<td>CH₃(CH₂)₁₃CH=CH(CH₂)₁₁COOH</td>
</tr>
<tr>
<td>unsaturated</td>
<td>Docosapentaenioic (22:5)</td>
<td>CH₃CH₂CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₃COOH</td>
</tr>
<tr>
<td>Unsaturated</td>
<td>Docosahexaenoic (22:6)</td>
<td>$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}$(CH$_2$)$_2$COOH</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Saturated</td>
<td>Lignoceric (24:0)</td>
<td>$\text{CH}_3-(\text{CH}<em>2)</em>{22}-\text{COOH}$</td>
</tr>
</tbody>
</table>

The most common FAs in edible oils have 18 carbon atoms. According to Table 2.17, these FAs include stearic (saturated), oleic (one double bond), linoleic (two double bonds) and linolenic (three double bonds) and Octadecatetraenoic (four double bonds) acids.

2.13 GAS CHROMATOGRAPH ANALYSIS OF MICROALGAE BIODIESEL

Figure 2.10 shows the fatty acid profile of lipid extracted from Tetraselmis suecica during early stationary phase (Halim et al., 2012). Tetraselmis suecica is a common green microalgae and their fatty acid profile illustrates the suitability of microalgae lipids for biodiesel production. Having C16:0, C18:1 and C18:2 as its principal fatty acids, Tetraselmis suecica lipid appears to have the required fatty acid profile for conversion to high-quality biodiesel. Saturated fatty acid content (27.6%) is relatively low when compared to the total cis-unsaturated fatty acid content (71.6%). This is desirable as FAME derived from cis-unsaturated fatty acids often has advantageous cold flow properties.
Figure 2.10 (a): Fatty acid composition of crude lipid extraction from Tetraselmis suecica algae species at the end of logarithmic phase (the beginning of the stationary phase) in terms of fatty acid chain. The letter t after the fatty acid name denotes trans-isomerism. When no letter t appears, fatty acid is of cis-isomerism. (Halim et al., 2012)

Laurens et al. (2012) reported the FAME yield of the in-situ transestrification of different species of algae using catalyst combination HCl/MeOH. The FAME were analyzed by GC-FID (Agilent 6890N, HP 5-MS column (Agilent, USA), 30 m, 0.25 mm ID and 0.25 μm FT, temperature program 70-300 °C, plateau for 1 min at 230 °C, at a 1.5 mL min⁻¹
He constant carrier gas flow. Quantification of the FAMEs was based on integrating the area under individual fatty acid peaks in the gas chromatograms. Then it was quantified using a 5-point calibration curve prepared with the standard of each FAME.

Table 2.18 shows their results of the total FAME profile of Nannochloropsis sp., Chlorella vulgaris replete, and Chlorella vulgaris deplete biodiesel resulted from in-situ transestrification using HCl/MeOH

Table 2.18: The FAME profile for three biomass algae in-situ transestrified using HCl/MeOH (Laurens et al. 2012)

<table>
<thead>
<tr>
<th>FAME</th>
<th>Nannochloropsis</th>
<th>Chlorella vulgaris replete</th>
<th>Chlorella vulgaris deplete</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>4.49±0.03</td>
<td>0.16±0.02</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td>C16:3</td>
<td>0.8±0.02</td>
<td>0.31±0.31</td>
<td>ND</td>
</tr>
<tr>
<td>C16:4</td>
<td>0.93±0.01</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C16:2</td>
<td>ND</td>
<td>6.05±0.11</td>
<td>2.45±0.07</td>
</tr>
<tr>
<td>C16:1</td>
<td>37.17±0.21</td>
<td>12.34±0.8</td>
<td>8.69±0.18</td>
</tr>
<tr>
<td>C16:0</td>
<td>19.98±0.1</td>
<td>18.33±0.33</td>
<td>18.04±0.49</td>
</tr>
<tr>
<td>C18:2</td>
<td>2.32±0.02</td>
<td>15.11±0.26</td>
<td>6.7±0.09</td>
</tr>
<tr>
<td>C18:1</td>
<td>3.01±0.03</td>
<td>18.91±2.11</td>
<td>61.9±0.96</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.5±0.02</td>
<td>23.99±1.57</td>
<td>ND</td>
</tr>
<tr>
<td>C18:0</td>
<td>0.41±0.02</td>
<td>1.18±0.03</td>
<td>1.28±0.06</td>
</tr>
<tr>
<td>C20:4</td>
<td>4.47±0.04</td>
<td>0.01±0.01</td>
<td>ND</td>
</tr>
<tr>
<td>C20:5</td>
<td>23.13±0.24</td>
<td>0.2±0.04</td>
<td>ND</td>
</tr>
<tr>
<td>C20:0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.8±0.02</td>
<td>0.89±0.15</td>
<td>0.35±0.12</td>
</tr>
</tbody>
</table>

Each value is the FAME yield (% DW) of individual fatty acids together with the sum (Total) as the mean ± SD of the three replicate measurements. ND means not detected.

Fatty acid profiles determined by Gouveia et al., 2009 for different microalgae species are presented in Table 2.19. They showed that all microalgae lipids are mainly composed of unsaturated fatty acids (50-65%) and a significant percentage of Palmitic acid (C16:0) was also present (17-40%). These results agree with the present thesis findings. See Chapter 4 Section 4.7.3
Table 2.19: Main fatty acids present in different microalgae species: Spirulina maxima (sp), Chlorella vulgaris (cv), Scenedesmus (sc), Dunaliella tertiolecta (dt), Nannochloropsis sp. (nanno) and Neochloris oleabundans (neo) oil extracts. All results are given in grams of fatty acid per 100 g of dry algae. (Gouveia et al., 2009)

<table>
<thead>
<tr>
<th>Fatty Acid, g per 100 g of dry algae</th>
<th>Sp</th>
<th>Cv</th>
<th>Sc</th>
<th>Dt</th>
<th>Nanno</th>
<th>Neo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>41.74</td>
<td>28.56</td>
<td>23.71</td>
<td>18.17</td>
<td>30.96</td>
<td>20.76</td>
</tr>
<tr>
<td>Unsaturated</td>
<td>51.97</td>
<td>51.91</td>
<td>54.66</td>
<td>63.14</td>
<td>59.20</td>
<td>64.60</td>
</tr>
<tr>
<td>Total= Saturated + Unsaturated</td>
<td>93.71</td>
<td>80.47</td>
<td>78.37</td>
<td>81.31</td>
<td>90.16</td>
<td>85.36</td>
</tr>
</tbody>
</table>

Mallick et al., 2012 studied the fatty acid analysis of Chlorella vulgaris biodiesel using gas chromatography-mass spectrometry (GC-MS). Capillary column (30 m x 0.25 mm x 0.25 μm) and Methylpentadecanoate (C_{16}H_{32}O_{2}) as an internal standard were used for the analysis. Table 2.20 presents the fatty acid content of Chlorella vulgaris oil after transestrification. Major contents were palmitic (C_{16}:0) followed by stearic (C_{18}:0), linolenic (C_{18}:2) and oleic (C_{18}:1) acid methyl esters. The saturated fatty acids, i.e., palmitic and stearic, constitute almost 82% of biodiesel while esters of long chain unsaturated fatty acid such as linoleic acid were present in low quantities.

Table 2.20: Composition and relative percentage of fatty acid methyl esters in Chlorella vulgaris (Mallick et al. 2012)

<table>
<thead>
<tr>
<th>Fatty acid methyl ester</th>
<th>Molecular formula</th>
<th>Retention time (min)</th>
<th>Relative percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid (C_{16}:0)</td>
<td>C_{17}H_{34}O_{2}</td>
<td>10.85</td>
<td>62.4</td>
</tr>
<tr>
<td>Stearic acid (C_{18}:0)</td>
<td>C_{19}H_{38}O_{2}</td>
<td>11.58</td>
<td>19.5</td>
</tr>
<tr>
<td>Linoleic acid (C_{18}:2)</td>
<td>C_{19}H_{34}O_{2}</td>
<td>11.61</td>
<td>9.8</td>
</tr>
<tr>
<td>Oleic acid (C_{18}:1)</td>
<td>C_{19}H_{36}O_{2}</td>
<td>11.69</td>
<td>8.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>
The table points out a challenge in the lipids analysis in Chlorella Vulgaris and other algae intended for biodiesel production. The retention times of the C18 components are so close together that it may not be possible to obtain the individual percentage of each of the C18 fatty acid methyl ester. As explained in Chapter 4, this difficulty was encountered in the present work.

Figure 2.11 shows an example of gas chromatogram of Chlorella vulgaris biodiesel designed by Francisco et al. (2010). Lipids were extracted using Bligh and Dyer method (Bligh and Dyer, 1959). Then, conventional transestrification was carried out by adding 250 mg of algae oil to 5.0 ml of 0.5 mol L⁻¹ of NaOH in methanol. Fatty acid composition was determined using a VARIAN 3600 CX gas chromatograph. Their results showed that the predominant fatty acids for Chlorella vulgaris were heptadecenoic (C17:1) and pentadecanoic (C15:0) with dry weight percentages of 31.64% and 31.81% respectively.

Figure 2.11: Gas chromatogram Chlorella vulgaris biodiesel (Francisco et al., 2010)
2.14 EFFECT OF FAME COMPOSITION ON BIODIESEL PROPERTIES

The properties of biodiesel are mainly determined by its fatty acid esters properties, which are influenced by their structural features such as chain length, degree of unsaturation, and the branching of the chain. Mallick et al., 2012 demonstrated that biodiesel from Chlorella vulgaris contains mainly saturated fatty acid (roughly 82% of saturated fatty acid methyl esters) as shown in Table 2.20. Poly unsaturated fatty acids with four or more double bonds are common in algae biodiesel. These double bonds are exposed to oxidation during storage, thus reduce the ability of microalgae oil to produce biodiesel. Therefore, high content of saturated fatty acids resulted in high oxidative stability of the Chlorella vulgaris biodiesel produced (Mallick et al., 2012, Refaat, 2009). Iodine value is also a measure of total unsaturation within a mixture of fatty acids. Chlorella vulgaris biodiesel has a suitably low Iodine value and within the limits of European (EN) and Indian (IS) standards. Table 2.21 shows a comparison of Chlorella vulgaris biodiesel with petroleum diesel and various biodiesel standards.

Table 2.21: Comparison of Chlorella vulgaris biodiesel with petroleum diesel and various biodiesel standards (Mallick et al., 2012)

<table>
<thead>
<tr>
<th>Property</th>
<th>Biodiesel from Chlorella vulgaris</th>
<th>Petroleum diesel</th>
<th>Biodiesel standards</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ASTM 14214</td>
</tr>
<tr>
<td>Density at 15 °C (kg m⁻³)</td>
<td>881</td>
<td>850</td>
<td>-</td>
</tr>
<tr>
<td>Viscosity at 40 °C (mm² s⁻¹)</td>
<td>4.5</td>
<td>2.6</td>
<td>1.9-6.0</td>
</tr>
<tr>
<td>Calorific value (MJ kg⁻¹)</td>
<td>38.4</td>
<td>42.2</td>
<td>-</td>
</tr>
<tr>
<td>Iodine value (g I₂/100 g)</td>
<td>56.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acid value (mg KOH g⁻¹)</td>
<td>0.6</td>
<td>0.4</td>
<td>&lt;0.8</td>
</tr>
<tr>
<td>Cetane index</td>
<td>54.7</td>
<td>49-55</td>
<td>≥47</td>
</tr>
<tr>
<td>Ash content (%)</td>
<td>0.01</td>
<td>0.01</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>0.03</td>
<td>0.02</td>
<td>&lt;0.03</td>
</tr>
</tbody>
</table>
Chuck et al. 2009 studied the effect of the fatty acid methyl ester chain length and level of unsaturation on the Cetane number and melting point. These trends are shown in Figure 2.12. For example, Cetane number of pure methyl oleate (C18:0) is much higher than that of methyl linoleate (C18:2), as is the heat of combustion. The Cetane number represents the ignition delay of the fuel and therefore has an impact on the engine combustion process. Higher Cetane numbers are desirable. The melting point represents the use of the fuel at low temperatures. Improving cold-temperature flow characteristics requires a fuel with low saturated fatty acid level (Refaat, 2009).
Moreover, Unsaturation has a significant effect on NO\textsubscript{x} emissions. NO\textsubscript{x} emissions are higher (undesirable) on combustion of methyl linoleate (C18:2) than those of methyl oleate (C18:0) or methyl palmitate (C16:0) (Chuck et al. 2009, Refaat, 2009). Other important physical properties of biodiesel such as viscosity and density are directly related to the fatty acid profile of the biodiesel present. Viscosity of biodiesel increases with increasing the chain length but decreases with increasing unsaturation. The density
of fatty acids decreases with an increase of the carbon chain length, whereas highly
unsaturated samples are denser than more saturated samples. (Chuck et al. 2009). All
these correlations between the fatty acid profile and the biodiesel properties are so
important to understand. Thus, the composition of the oils and the alcohol used can both
be selected to produce biodiesel of optimal performance.

2.15 AVIATION TURBINE FUEL (JET FUEL)

British Petroleum (Clark, 2008) and Boeing (Paisly, 2008) estimate about 2 billion
barrels of jet fuel used in civil aviation worldwide per year. This amounts to about 230
million gallons jet fuel used worldwide per day. According to the Air Transport
Association (ATA), commercial airlines in the U.S. purchased about 18.85 billion gallons
of jet fuel in 2008. This translates to about 51.6 million gallons of jet fuel are used in the
US per day, or about 22.4% of the worldwide use.

The alternative and renewable bio-jet fuel of this study will be a blend of biodiesel and jet
fuel. It is possible that the bio-jet fuel blend does not fulfill all specifications. Table 2.22
Lists the essential requirements of bio-jet fuel that have to be always satisfied are (Clark,
2008 and Paisly, 2008) and the role of the present work.
Table 2.22: Essential requirements of bio-jet fuel and how addressed in the present work

<table>
<thead>
<tr>
<th>Essential Bio-jet Fuel Requirement</th>
<th>Role of Present Work</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintain performance over wide temperature range (-50 &lt; T (°C) &lt; +40).</td>
<td>Freezing point of bio-jet blend is evaluated</td>
</tr>
<tr>
<td>Maintain performance over wide pressure range (0.3 &lt; P (atm) &lt; 1).</td>
<td></td>
</tr>
<tr>
<td>Offer good energy content per unit weight.</td>
<td>Heating Value of bio-jet blend is evaluated</td>
</tr>
<tr>
<td>Permit easy handling and storage.</td>
<td>The fatty acids profile of the bio-jet blend is evaluated. Higher saturated fatty acids content improve long-term storage.</td>
</tr>
<tr>
<td>Be readily available on a global scale</td>
<td>Microalgae is readily available</td>
</tr>
<tr>
<td>Be cost competitive to fossil fuels</td>
<td>Use waste water to cultivate microalgae</td>
</tr>
</tbody>
</table>

2.16 BIOREFINERIES FOR SUSTAINABLE BIO-JET FUEL

The development of a more sustainable and economically feasible bio-jet process requires the use of all microalgae components (e.g., proteins, lipids, carbohydrates). This is the purpose of a biorefinery. Biorefinering is the production of a wide range of chemicals and bio-fuels from biomasses (e.g., microalgae). This is done by integrating bio-processing and low environmental impact chemical technologies in a cost-effective and environmentally sustainable (Vanthoor-Koopmansa 2013). Application of biorefining of microalgae requires the fractionation of algae into

- Lipids for biodiesel
- Lipids as a feedstock for the chemical industry and essential fatty acids
- Proteins and carbohydrates for food, feed and bulk chemicals.

Major advantages of algae biorefineries include:

- Use of industrial waste streams as inputs, e.g., CO2, wastewater and desalination plant rejects
- Production of several fuels (biodiesel, methane, ethanol and hydrogen) and non-energy derived (nutraceutical, fertilizers, animal feed and other bulk chemicals) products.

- Not competing with food production (non-arable land and no freshwater requirements)

The large-scale production of microalgae bio-jet fuel in biorefineries will become economically, environmentally and ethically extremely attractive (Ben-Hamadou, 2012).

2.17 BLENDING JP5 JET FUEL WITH BIODIESEL AND DIESEL FUELS

Korres et al. (2008) evaluated the performance and emissions of JP5 jet fuel along with its mixture in biodiesel and diesel fuel in a stationary diesel engine. The properties of the fuels are shown in Table 2.23. For the engine tests, the fuels were used alone and in various mixtures. The automotive diesel fuel was used as reference fuel.

Table 2.23: Properties of petroleum diesel, JP5 and biodiesel used in the experiment (Korres et al., 2008)

<table>
<thead>
<tr>
<th>Property</th>
<th>Automotive diesel</th>
<th>JP-5</th>
<th>Biodiesel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density @15°C, kg/l</td>
<td>0.8326</td>
<td>0.8145</td>
<td>0.8757</td>
</tr>
<tr>
<td>Sulfur Content, wt%</td>
<td>0.0045 wt%</td>
<td>0.0110 wt%</td>
<td>15 ppm w</td>
</tr>
<tr>
<td>Flash point, °C</td>
<td>65</td>
<td>68</td>
<td>&gt;120</td>
</tr>
<tr>
<td>Cetane number</td>
<td>55</td>
<td>50</td>
<td>56</td>
</tr>
</tbody>
</table>

JP-5 can potentially result in pump wear problems. However, biodiesel addition to the fuel definitely improves this situation, as will happen with Cetane number of JP-5, which is somewhat lower than the Cetane number of diesel fuel. The ternary mixtures showed the following behavior, with NOx emissions initially reduced with increasing JP-5 content by volume and increased for increasing biodiesel content by volume.
This increase is attributed to the availability of oxygen content in the biodiesel, which leads to better oxidation of the nitrogen available, thus increasing the emissions of nitrogen oxides. Therefore, biodiesel has clearly shown to provide increased NOx emission levels. The addition of biodiesel in diesel caused a large reduction in PM emissions and this can be attributed to the oxygen content of the fuel. Biodiesel addition to JP-5 also reduced PM emissions as compared to the JP-5 alone and this is attributed to the higher Cetane number of biodiesel and improved combustion efficiency. Biodiesel increased the fuel consumption when added to petroleum fuels and the increase was larger at high engine loads. This is attributed to the oxygen content of biodiesel. The ternary blends with lower biodiesel content by volume, showed lower volumetric fuel consumption. The biodiesel increased volumetric fuel consumption due to its chemically bound oxygen content. In contrast, the petroleum derived fuels showed about the same consumption results. The addition of biodiesel in small quantities is expected to improve certain properties that are of significant importance for diesel engines.

2.18 LITERATURE REVIEW SUMMARY AND CONCLUSION

A brief summary of the literature review presented in this Chapter is given in Table 2.24

<table>
<thead>
<tr>
<th>Research area</th>
<th>Cited work</th>
<th>Comment/Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalgae Cultivation</td>
<td>Ben-Amotz, 2007, Chen et al., 2011, Barsanti 2006</td>
<td>Cultivation Methods, biomass productivity, lipid content</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Lipid Triggering</td>
<td>Converti et al., 2009, Gouveia, et al., 2009, Lv et al., 2010, Mallick et al., 2012</td>
<td>Applied to increase the FAME production.</td>
</tr>
<tr>
<td>Algae Harvesting</td>
<td>Chen et al., 2011, Elmoraghy et al., 2012, Webster, 2010, Halim et al., 2012, Brennan et al., 2010</td>
<td>Dewater microalgae solution to obtain dry algae required for lipid extraction</td>
</tr>
<tr>
<td>Method</td>
<td>Authors/Techniques</td>
<td>Description</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Conventional Transesterification</td>
<td>Wcislo, 2008, Rajan et al., 2010, Laurens et al., 2012,</td>
<td>Two-step biodiesel production</td>
</tr>
<tr>
<td>Gas Chromatograph Analysis of Microalgae Biodiesel</td>
<td>Halim et al., 2012, Laurens et al., 2012, Gouveia et al., 2009, Mallick et al., 2012, Bligh and Dyer, 1959, Francisco et al., 2010</td>
<td>Identification of biodiesel FAMEs</td>
</tr>
<tr>
<td>Effect of FAME Composition of Biodiesel Properties</td>
<td>Mallick et al., 2012, Refaat, 2009, Chuck et al., 2009,</td>
<td>Determine the properties of the biodiesel produced</td>
</tr>
<tr>
<td>Blending Jet fuel with Biodiesel</td>
<td>Korres et al., 2008</td>
<td>To obtain bio-jet fuel</td>
</tr>
</tbody>
</table>

The literature review supports the objectives of this research. For microalgae biodiesel there is a need to

- Investigate the use of LEDs to minimize energy requirements for microalgae growth and CO2 emission
- The effects of light intensity
- Nitrogen starvation
- Scale-up on algae oil production
- The FAME distribution
- Demonstrate that municipal waste water can be used in growing microalgae to reduce fresh water use
- Develop a kinetics model of microalgae growth in batch reactor
- Evaluate the effectiveness of the in-situ process to improve the processing time and cost.

For bio-jet fuel there is a need to
- Investigate biodiesel blending with jet-fuel in order to obtain bio-jet fuel
- Evaluate the properties of the bio-jet product.

These are addressed in this investigation.

The next Chapter will discuss the Experimental Methods.
CHAPTER III

3 EXPERIMENTAL PROCEDURES

3.1 MEASUREMENTS, METRICS AND INSTRUMENTS

The metrics are intended to provide measuring units to establish the status of the project and realize the targets that have been achieved. The measurement techniques were divided into several types based on the metrics task to be completed/accomplished. These are given in Table 3.1.

Table 3.1: Project metrics and measurements

<table>
<thead>
<tr>
<th>Metric</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Algae Growth</td>
<td>Monitor turbidity readings and cell counts. The higher the algae growth, the darker is the solution and the higher are the turbidity and the number of algae cells counted in a known volume of solution.</td>
</tr>
<tr>
<td>2-Water Quality</td>
<td>Monitor the nutrient concentration and pH during algae growth. It is important to track how the algae are growing so they are harvested when the maximum amount of algae are present.</td>
</tr>
<tr>
<td>3-Process Efficiencies</td>
<td>Record light intensity and air flow rate measurements to evaluate photosynthetic efficiency and the carbon sequestration efficiency.</td>
</tr>
<tr>
<td>4-Algae Biomass Production</td>
<td>After harvesting the algae were dewatered, freeze dried. The algae production per volume of solution was determined gravimetrically.</td>
</tr>
<tr>
<td>5-Algae Oil Production</td>
<td>The algae oil is extracted using hexane solvent. The oil production per unit volume of solution was determined gravimetrically.</td>
</tr>
<tr>
<td>6-LED Energy Minimization.</td>
<td>Replace fluorescent lights with light emitting diodes (LEDs). Repeat steps 1-4. Compare algae biomass and oil production.</td>
</tr>
<tr>
<td>7-Municipal waste water use.</td>
<td>Replace fresh water with municipal waste water. Repeat steps 1-4. Compare algae biomass and oil production.</td>
</tr>
<tr>
<td>8-FAMEs composition</td>
<td>Gas chromatograph analysis is used to identify and quantify the FAMEs in order to determine the properties of biodiesel</td>
</tr>
</tbody>
</table>
Specific gravity, freezing point and heat of combustion of the blend with different ratios were determined.

The measured variables, instruments and the metrics calculated of this work are listed in Table 3.2.

Table 3.2: The measured variables, the instruments and the metrics used in the present study

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Instrument</th>
<th>Purpose/ metrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily algae solution turbidity</td>
<td>Bausch and Lomb Spectrophotometer</td>
<td>Algae growth, from turbidity measurement at 682 nm.</td>
</tr>
<tr>
<td>Daily algae solution cell count</td>
<td>Microscope with hemocytometer</td>
<td>Algae growth, in cells/ml.</td>
</tr>
<tr>
<td>Acidity of solution (pH), nitrite and nitrate levels</td>
<td>Mardel 5 in 1 test strips</td>
<td>PH measurements, nutrient concentration and if there is nutrient depletion or starvation.</td>
</tr>
<tr>
<td>Algae mass after harvesting and drying</td>
<td>Balance</td>
<td>Algae production expressed in final concentration, (g dry algae/L)</td>
</tr>
<tr>
<td>Mass of algae Oil after extraction and hexane evaporation.</td>
<td>Balance as above</td>
<td>Algae oil yield (g oil/100 g dry algae)</td>
</tr>
<tr>
<td>Air flow rate, liters/min</td>
<td>Rotameter</td>
<td>Carbon sequestration efficiency</td>
</tr>
<tr>
<td>Incident light intensity</td>
<td>Extech light meter</td>
<td>Photosynthetic efficiency</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>FAME composition</td>
<td>Hewlett Packard HP 5890 Series II Gas chromatograph</td>
<td>Transestrification FAME yield (Biodiesel production)</td>
</tr>
<tr>
<td>Specific gravity of the blend</td>
<td>Balance and graduate cylinder</td>
<td>Determine the mass and the volume of each sample to determine the density</td>
</tr>
<tr>
<td>Heat of combustion of the blend</td>
<td>Bomb calorimetry</td>
<td>Heating value of fuel</td>
</tr>
<tr>
<td>Freezing point of the blend</td>
<td>Digital thermometer</td>
<td>Determine the temperature at which the fuel or the blend will stay liquid.</td>
</tr>
</tbody>
</table>
3.2 MATERIALS AND REAGENTS

The materials used during the processes of this work and their functions are listed in Table 3.3.

Table 3.3: Materials used for this work and their functions

<table>
<thead>
<tr>
<th>Material</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>PETCO air stone (6 inches length)</td>
<td>Bubble air into the medium and insure uniform air distribution in the 2L PBR</td>
</tr>
<tr>
<td>Long flexible plastic tubes 2 mm ID</td>
<td>Supply air into the 2L PBRs as they are connected to the air stone.</td>
</tr>
<tr>
<td>Long glass tubes 0.5 mm ID, 48 in length</td>
<td>Supply air bubbles into the 80 L PBR</td>
</tr>
<tr>
<td>Eppendorf centrifuge 5810R</td>
<td>Separate algae cells from the medium.</td>
</tr>
<tr>
<td>ICE B-20 A centrifuge</td>
<td>Dry algae cells</td>
</tr>
<tr>
<td>Labconco freeze dryer 5</td>
<td>Dry algae cells</td>
</tr>
<tr>
<td>Lipid extraction apparatus (250 ml glass flask+ Pyrex condenser)</td>
<td>Extract algae lipids</td>
</tr>
<tr>
<td>Buchner funnel</td>
<td>Extract algae lipids</td>
</tr>
<tr>
<td>0.25 µm Whatman filter paper grade # 5</td>
<td>Separate the solid from the liquid</td>
</tr>
<tr>
<td>Water bath</td>
<td>Evaporate the solvent</td>
</tr>
<tr>
<td>Oven</td>
<td>Dry the samples or the oil removing any moisture</td>
</tr>
<tr>
<td>Hot plate with magnetic stirrer</td>
<td>Heat the solution and mix its contents</td>
</tr>
<tr>
<td>Magnetic bars</td>
<td>Mix the solution</td>
</tr>
<tr>
<td>Ultrasonic W375 Sonicator</td>
<td>Break down algae cell membrane to release the oil</td>
</tr>
<tr>
<td>Digital micro pipette 20 200 µl</td>
<td>Measure small volume to prepare cocktail standard</td>
</tr>
<tr>
<td>2 ml glass vial</td>
<td>To store cocktail standard</td>
</tr>
<tr>
<td>20 ml glass vial</td>
<td>To store the standards and the mix of biodiesel sample and chloroform</td>
</tr>
<tr>
<td>5 µL Hamilton syringe</td>
<td>Inject the sample in the GC</td>
</tr>
<tr>
<td>Restek RTX-1 column: 30 m, 3 µm df, 0.32 mm ID</td>
<td>Helium carrying the FAMEs pass through the column</td>
</tr>
<tr>
<td>HP3396 Integrator</td>
<td>Convert the GC data into a chromatogram</td>
</tr>
<tr>
<td>150 ml glass beaker</td>
<td>Use for sonication process</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>250 ml glass flask</td>
<td>Use for filtration</td>
</tr>
<tr>
<td>125 ml glass flask</td>
<td>Use for evaporation</td>
</tr>
<tr>
<td>Glass graduate cylinders</td>
<td>Measure the volume of the sample</td>
</tr>
<tr>
<td>Fluorescent lamps</td>
<td>Illuminate the PBRs</td>
</tr>
<tr>
<td>Red LEDs panels</td>
<td>Illuminate the PBRs</td>
</tr>
<tr>
<td>Red-Blue LEDs panel</td>
<td>Illuminate the PBRs</td>
</tr>
<tr>
<td>Reflective Mylar sheets</td>
<td>For LEDs jacket design, this used to illuminate the 80 L PBR, to reflect any unabsorbed light back into the algae solution.</td>
</tr>
<tr>
<td>Wide Velcro</td>
<td></td>
</tr>
<tr>
<td>Flexible water proof LED strips (available in red and blue colors)</td>
<td>For LEDs jacket design which used to illuminate the 80 L PBR.</td>
</tr>
</tbody>
</table>

Reagents used through this work and their functions are listed in Table 3.4.

Table 3.4: Reagents and their functions
(note: the nutrients used for algae growth are not included)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum Sulfate</td>
<td>As a flocculent to settle the algae while harvesting</td>
</tr>
<tr>
<td>Sodium Sulfate</td>
<td>Removing moisture from biodiesel during filtration</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Preservative for FAMEs</td>
</tr>
<tr>
<td>Potassium Hydroxide</td>
<td>Base catalyst for transesterification process</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>Cleaning purposes</td>
</tr>
<tr>
<td>Acetone</td>
<td>Cleaning purposes of the glass ware</td>
</tr>
<tr>
<td>Methanol</td>
<td>For transesterification process</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Cleaning purposes of the glass ware</td>
</tr>
<tr>
<td>Hexane</td>
<td>As a solvent for lipid extraction</td>
</tr>
<tr>
<td>Jet fuel</td>
<td>For blending with biodiesel</td>
</tr>
<tr>
<td>B100 Biodiesel from waste vegetable oil (WVO)</td>
<td>For blending with jet fuel</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>As a standard for the heat of combustion calculation</td>
</tr>
<tr>
<td>Reverse Osmosis water</td>
<td>Algae growth medium</td>
</tr>
<tr>
<td>Waste water</td>
<td>Algae growth medium</td>
</tr>
<tr>
<td>Methyl Palmitate C16:0</td>
<td>GC standards for FAME analysis</td>
</tr>
<tr>
<td>Methyl Stearate C18:0</td>
<td></td>
</tr>
<tr>
<td>Methyl Oleate C18:1</td>
<td></td>
</tr>
<tr>
<td>Methyl Linoleate C18:2</td>
<td></td>
</tr>
<tr>
<td>Methyl Linolenate C18:3</td>
<td></td>
</tr>
</tbody>
</table>
3.3 ALGAE SPECIES SELECTED AND GROWTH CONDITIONS

Two different species of microalgae were used in this work; Chlorella vulgaris and Chlorella salina (simply termed Chlorella C2). Two different species of microalgae were used in this work; Chlorella vulgaris and Chlorella salina (simply termed Chlorella C2). These species were selected because they are single-cell green algae that grow fast, are very resilient and can be grown both in fresh or reverse osmosis (R.O.) water and in wastewater. Each species was inoculated in the Photosynthesis Laboratory at the UNH Plant Biology Department. 5 ml of each of these inoculations was cultured in 2L glass flask in the Biodiesel laboratory. The growth medium in the flask contained the required nutrients for each species to grow. The nutrients included macronutrients and micronutrients. The composition of the nutrient solution is listed in Table 3.4, Section 3.7.1. The temperature was between 25°C and 27°C. Lighting (fluorescent or LED) and aeration were provided continuously throughout the growing phase. The growth of each culture was monitored until it reached the stationary phase. Then these cultures were harvested after 8 to 10 days growth period and kept refrigerated to be used along this work.

3.4 PHOTOBIOREACTOR (PBR) DESIGN

Two types of PBRs were used for this study; 2L for small scale and 80 L for large scale.

3.4.1 SMALL SCALE PBR

The algae cultivation setup consisted of four liter clear plastic photobioreactors (obtained from PETCO a fish supply company). Each reactor has the dimensions; length=
16.5 in, width = 3.4 in and height = 12.2 in. Each reactor was divided into two separate 2 L PBRs and operated at the same time in the Biodiesel Lab. This insured that the two adjacent 2L PBRs were exposed to the same light photonic energy. Figure 3.1 show the 4L fish tank

![4L fish tank divided into two separate 2L PBR.](image)

3.4.2 LARGE SCALE PBR

The large scale PBR is 80 L liter cylindrical PBR made of clear plastic to enhance light penetration. It has dimensions of 30 cm diameter and 120 cm height. A valve at the bottom of the cylindrical PBR is used to transfer algae solution to four 5-gallon carboys (see Appendix I). Figure 3.2 shows the 80 L cylindrical PBR.
3.4.3 AIR SUPPLY

The air source was a compressed air line in the Biodiesel Lab. The compressed air was passed through a simple trap to remove oil that might be in the compressed air line. The air was introduced and bubbled into the 2 L PBR using an air stone (PETCO, 6 inches length) to insure uniform air distribution (See Appendix I). Air flow rate was adjusted to accomplish mixing the content of the PBR. Air contained about 392 ppm of CO2, which was sufficient to provide the inorganic carbon needed for photoautotrophic growth. The air was introduced to the 80 L PBR as air bubbles using 3 glass tubes with 5 mm outside diameter, 1 mm inside diameter and 100 cm length. Air flow rate was maintained at 3.6 L/min.
3.5 LIGHT ENERGY SOURCE

Three light sources were used to study the lighting effect on Chlorella vulgaris and Chlorella C2 algae growth and lipid production. These are fluorescent light, red LEDs and red-blue LEDs. These light sources are shown in Appendix I.

3.5.1 FLUORESCENT LIGHT

Eight daylight fluorescent lamps are used to illuminate the 2 L and 80 L PBRs, have power consumption of 68 Watt.

3.5.2 RED LED LIGHTS

Three red LEDs panels surround the 4L fish tank from three sides with a total power consumption of 33 Watt. Each panel is 30 X 30 cm and has 225 LEDs.

3.5.3 RED-BLUE LED LIGHTS

Three red-blue LEDs panels surround the 4L fish tank from three sides with a total power consumption of 35 Watt. Each panel is 30 X 30 cm and has 165 red LEDs and 60 blue LEDs, so the ratio of the red: blue LEDs is about 3:1.
3.5.4 LIGHT INTENSITY MEASUREMENTS

There are several units to measure the light intensity. The most important two are the LUX and micromole/m²-s. The LUX is the SI unit of illuminance. It measures luminous flux per unit area. It is also a measure of the intensity, as perceived by the human eye, of light that hits or passes through a surface. The human eye is most sensitive to light at 555 nm (green) than any other wavelength. This means that the highest LUX for a given electric power flux (W/m²) occurs at 555 nm. For other wavelengths of visible light, e.g., red or blue, lower LUX will be measured for the same electric power flux. The luminosity function, or LUX, becomes zero for wavelengths outside the visible spectrum.

The micromole/m².s is the Photosynthetic Photon Flux (PPF). It expresses the light quantum in photons of solar radiation from 400 to 700 nm (visible light) that photosynthetic organisms are able to use in photosynthesis. Photons at shorter wavelengths tend to be so energetic that they can be damaging to cells and tissues. Photons at longer wavelengths do not carry enough energy to allow photosynthesis to take place. The most abundant plant pigment is Chlorophyll. It is very efficient in capturing red and blue light.

3.6 ALGAE GROWTH MEDIUM
One of the goals of this study is to minimize fresh water requirements for algae growth. To accomplish this goal, an inexpensive source of water and nutrients for the algae; municipal waste water; was used to grow microalgae. Microalgae biomass and oil production using fresh water and waste water were compared.

### 3.6.1 FRESH WATER CHARACTERISTICS

The fresh water used was obtained from a reverse osmosis (R.O.) unit available in Dr. Jahnke’s Lab in the Plant Biology Department.

### 3.6.2 WASTE WATER CHARACTERISTICS

The municipal waste water used was obtained from the Dover NH wastewater treatment plant. For safety purposes the wastewaster was collected after ultra-violet (UV) treatment. This insures that absence of pathogens (which can cause a variety of illnesses). Typical properties of the waste water used are pH=6.83, Nitrate nitrogen (NO$_3$) = 7.5 mg/l, Ammonia Nitrogen (NH$_3$) = 6 mg/l, Total nitrogen (TN) =14 mg/l, Total phosphate (TP) =1.3 mg/l, Biological oxygen demand (BOD) =10 mg/l, Total suspended solids (TSS) =7 mg/l. The nutrient solution used has 1.05 g KNO3 in 2 L= 525 mg KNO3/l. 1 g of KNO3 contains 0.139 g N. This KNO3 is equivalent to 72 mg N/l. The nutrient solution also has 0.1136 g Na3PO4 in 2 L. This is equivalent to 12.4 mg P/l. Hence, variation in the waste water effluent TN and TP will have very little effect on available N and P for algae growth. In terms of the carbon, the BOD value of 10 mg O$_2$/l is equivalent to 3.75 mg C per liter. The air flow rate was 3.6 L/min (see section 3.4.3). Based on 392
ppmv CO$_2$ the total carbon introduced into the growth solution over a growth period of 18 days was 17.96 g. The carbon content of the waste water is about 0.041% of the C in the air. Hence the effect of C in waste water on algae growth is negligible.

### 3.7 ALGAE GROWTH IN PBR

#### 3.7.1 GROWTH IN 2L PBR (SMALL SCALE)

Algae were grown in three fish tank, i.e., six 2L PBRS at the same time. Each two adjacent 2L PBRs were filled with two different medium; 2L of fresh water and 2L of waste water respectively (See appendix I). The first tank was surrounded with the red LEDs panels, the second fish tank was surrounded with the red-blue LEDs panels and the third fish tank was placed in front of the fluorescent light. These light sources provided photonic energy to the algae solution 24 hours a day for the entire growth period. The required nutrients listed in Table 3.5 were added to each PBR. It is important to note that the same nutrients were added to both; fresh water and waste water mediums. Air was continuously bubbled in the PBR to provide CO$_2$ to the algae solution during the growth period. Air was supplied through long plastic tubes connected to an air stone, which placed at the bottom of the each PBR.
Table 3.5: Required nutrients for algae growth

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Chemical formula</th>
<th>Molecular Weight, MW (g/gmol)</th>
<th>Concentration millimoles mM</th>
<th>Concentration (mg/l)</th>
<th>Mass added for 2L medium (mg)</th>
<th>Mass added for 80 L medium (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Chloride</td>
<td>CaCl₂•2H₂O</td>
<td>147</td>
<td>0.2</td>
<td>29.4</td>
<td>58.8</td>
<td>2,352</td>
</tr>
<tr>
<td>Boric acid</td>
<td>H₃BO₃</td>
<td>61.83</td>
<td>0.13</td>
<td>8.06</td>
<td>16.12</td>
<td>644.8</td>
</tr>
<tr>
<td>Potassium Nitrate</td>
<td>KNO₃</td>
<td>101.1</td>
<td>5.19</td>
<td>525.2</td>
<td>1050.4</td>
<td>42,016</td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
<td>MgSO₄</td>
<td>120.4</td>
<td>5</td>
<td>601.85</td>
<td>1203.7</td>
<td>48,148</td>
</tr>
<tr>
<td>Sodium Phosphate</td>
<td>Na₂HPO₄</td>
<td>141.96</td>
<td>0.39</td>
<td>56.8</td>
<td>113.6</td>
<td>4,544</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>NaCl</td>
<td>58.4</td>
<td>99.3</td>
<td>5800</td>
<td>11600</td>
<td>464,000</td>
</tr>
<tr>
<td>EDTA (Ethylene diamine Tetraacetic Acid)</td>
<td>C₁₀H₁₆N₂O₈</td>
<td>292.24</td>
<td>0.092</td>
<td>26.9</td>
<td>53.8</td>
<td>2152</td>
</tr>
<tr>
<td>Ferrous Sulfate</td>
<td>FeSO₄•7H₂O</td>
<td>278.01</td>
<td>0.01</td>
<td>2.8</td>
<td>5.6</td>
<td>224</td>
</tr>
<tr>
<td>Zinc Sulfate</td>
<td>ZnSO₄•7H₂O</td>
<td>287.56</td>
<td>0.001</td>
<td>0.288</td>
<td>0.576</td>
<td>23.04</td>
</tr>
<tr>
<td>Molybdenum Oxide</td>
<td>MoO₃</td>
<td>143.94</td>
<td>0.00087</td>
<td>0.125</td>
<td>0.25</td>
<td>10</td>
</tr>
<tr>
<td>Copper Sulfate</td>
<td>CuSO₄•5H₂O</td>
<td>249.68</td>
<td>0.0003</td>
<td>0.075</td>
<td>0.15</td>
<td>6</td>
</tr>
<tr>
<td>Cobalt Chloride</td>
<td>CoCl₂•6H₂O</td>
<td>237.93</td>
<td>0.0001</td>
<td>0.025</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>Manganese Chloride</td>
<td>MnCl₂•4H₂O</td>
<td>197.9</td>
<td>0.00076</td>
<td>0.15</td>
<td>0.3</td>
<td>12</td>
</tr>
</tbody>
</table>

The same nutrients listed in Table 3.4 are required for both Chlorella C2 and Chlorella vulgaris except that Chlorella vulgaris does not need any boric acid (H₃BO₃) to grow. After all the nutrients dissolved into the medium, Algae inoculum was added at a ratio of algae inoculum to medium by volume equal to 1:100 for the 2 L (small scale) PBRs. Thus, 20 ml of algae inoculum were added to 2L medium. Algae growth was monitored every other day through the growth period by measuring the turbidity of the algae.
solution using the spectrophotometer and the cell counts using the microscope. Some other important measurements were recorded during the growth period such as pH, nitrate concentration and nitrite concentration using Mardel test strips. The strips has a pH range of 6.2 - 8.4 and nitrate concentration range of 0 – 200 mg/l (ppm) and nitrite concentration range 0 – 10 mg/l (ppm).

3.7.2 GROWTH IN 80L PBR (LARGE SCALE)

For scale-up purpose, algae were grown in 80L cylindrical PBR with the same technique as in the 2L PBR except the following variations; air supplied to the 80 L PBR as air bubbles through glass tubes, red-blue LEDs jacket designed by Daniel Eltringham was used to illuminate the 80L PBR. The red-blue LEDs jacket construction is described in appendix I and 300 ml of algae inoculum were added for the 80L nutrient medium, i.e., the ratio algae inoculum: nutrient solution is 1:3.75.

3.7.3 CONTROL RUNS

It is important to make sure that the growth medium and air are not contaminated with bacteria or other algae species. This was done through a series of Control Runs, which are summarized in Table 3.6.
Table 3.6: Control experimental runs

<table>
<thead>
<tr>
<th>No.</th>
<th>Algae strain</th>
<th>Growth Medium</th>
<th>Air used</th>
<th>Light Source</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fresh water</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No Growth</td>
<td>Fresh water has no bacteria that would grow</td>
</tr>
<tr>
<td>2</td>
<td>Waste water</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No Growth</td>
<td>Waste water has no bacteria that would grow</td>
</tr>
<tr>
<td>3</td>
<td>Fresh water</td>
<td>Air bubbles</td>
<td>Fluorescent</td>
<td>No Growth</td>
<td>Fresh water has no bacteria that would grow in light or air</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Waste water</td>
<td>Air bubbles</td>
<td>Fluorescent</td>
<td>No Growth</td>
<td>Waste water has no bacteria that would grow in light or air</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>C2</td>
<td>Fresh water</td>
<td>No</td>
<td>No</td>
<td>No Growth</td>
<td>C2 requires air and light to grow in Fresh water</td>
</tr>
<tr>
<td>6</td>
<td>C2</td>
<td>Waste water</td>
<td>No</td>
<td>No</td>
<td>No Growth</td>
<td>C2 requires air and light to grow in Waste water</td>
</tr>
</tbody>
</table>

3.8 ALGAE HARVESTING

For optimum biomass productivity, the best time to harvest the algae is after reaching the stationary phase when the cell concentrations would remain relatively unchanged (Lv et al., 2010). This could be determined via turbidity and/or cell counts measurements. Then it was necessary to harvest the algae solution and separate the algae cells from the nutrient solution. This was done by centrifuging the algae samples. Two
different centrifuge devices were used for this work; a Damon/IEC B-20A centrifuge, which was operated at 5000 rpm for 20 minutes and Eppendorf centrifuge 5810R, which was operated at 4000 rpm for 20 minutes.

The centrifuge produced algae paste and a clear liquid. The clear liquid was discarded. The algae paste was shelled by placing in a Labcono jar and surrounded by a mixture of dry ice, acetone and methanol. This mixture was used to freeze the algae paste. Then, the frozen algae were freeze dried at -80°C under vacuum for about 48 hours using Labconco freeze dryer 5. The centrifuge and the freeze dryer devices were available at the UNH Dairy Research Center. The dried algae were massed (mass of freeze dried algae, g) and used in lipid extraction. The algae biomass production was calculated using equation 3.1

\[
\text{Algae biomass production, g/L Solution-day} = \frac{\text{(mass of freeze dried algae, g)}}{(\text{Total volume of algae solution, L}) \times (\text{growth period, day})}
\]

Equation 3.1

Note that total volume of algae solution is the volume after the algae growth reached the stationary phase and needed to be harvested.

It is impractical to use centrifugation to dewater the 80L batch; large volume centrifugation is labor and energy intensive (Chen et al., 2011). Therefore, a flocculent was added to the algae medium after growth in order to settle the algae cells at the bottom and leave a relatively clear liquid at the top. In this study, Aluminum sulfate anhydrous \( \text{Al}_2(\text{SO}_4)_3 \) was added at a concentration of 0.2 g of \( \text{Al}_2(\text{SO}_4)_3 \) per liter of medium solution. The total volume of algae solution at the end of the growth was about 70-72 L
due to evaporation. The algae solution was transferred to four 5-gallon carboys. Aluminum sulfate was added, and the carboys were vigorously agitated to ensure thorough mixing. After twelve hours, the algae settled to the bottom of the carboys. The clear top layer of liquid was removed by siphoning and discarded. Due to the reduction in volume, only 5-6 liters remain to be centrifuged from each 80L batch. This quantity can easily be centrifuged to complete dewatering.

3.9 ALGAE LIPID EXTRACTION (FLASK METHOD)

The lipid extraction process used in this study is based on the solvent extraction. The solvent used in this study is n-Hexane. The extraction apparatus consists of 250 ml glass flask connected to a Pyrex condenser. All the glass wares were cleaned before use for lipid extraction using the following reagents respectively: 0.1 M Hydrochloric acid, distilled water, acetone, methanol, and Hexane. Each reagent was added into the flask and swirled around and then disposed into the appropriate hazardous waste container. Freeze dried algae biomass was pulverized using mortar and pistol. 40 ml of the solvent; normal Hexane (n-hexane) poured into 250 ml glass flask. 1 g of pulverized dry algae were massed and added to the solvent. Magnetic bar was added. The assembled apparatus is shown in Figure 3.3. The condenser is then connected to cold tap water to ensure the circulation of cold water through the condenser. The lipid extraction apparatus was placed on a hot plat with magnetic stirrer. The temperature was set at 70°C (boiling point of n-Hexane) and the stirrer was set at 1 degree to enhance mixing the solution. After two hours of operation, the hot plat was turned off to allow the glassware to cool down to
room temperature. Then, the condenser and the flask were separated carefully. The mixture of algae oil and hexane was filtered using 0.25 μm Whatman filter paper grade #5 and a Buchner funnel to remove the algae particles after extracting the lipids. The filtration was repeated two times at least to ensure removing the solids. The collected lipids with the solvent was transferred into 125 ml glass flask [mass of the 125 ml flask was recorded as the mass of (the empty flask), g] and was placed in a water bath set at 40°C to evaporate the solvent (n-hexane). In addition, air was supplied to the flask that has the lipids with the solvent to speed the evaporation. Once all the solvent evaporated, the 125 ml glass flask contained only the lipids was put in the oven set at 40°C for one hour in order to complete the drying. Then, the flask was allowed to cool down to room temperature. The mass of the flask that has the lipids was weighed [the mass of (flask + lipid), g]. The difference in mass of [the mass of (flask + lipid), g] and [the mass of (the empty flask), g] give the mass of the extracted crude lipids. The lipid yield was determined using Equation 3.2

\[
\text{Lipid Yield} = \frac{\text{mass of (flask+lipid)} - \text{mass of (empty flask)}}{\text{mass of dry algae}} \times 100
\]

Equation 3.2
3.10 TRANSESTRIFICATION

3.10.1 CONVENTIONAL TRANSESTRIFICATION

The conventional transestrification process to produce biodiesel, also termed as two-step process, consists of two steps; the first step is to extract the lipids from algae cells and the second step is to convert the extracted lipids into biodiesel through transestrification reaction described in Reaction (1.1).

After lipid extracted using flask method as described in Section 3.9, the evaporation of the solvent should be stopped when 1 ml of the solvent (n-hexane) was left mixed with the lipids. Then 25 ml of 0.1 KOH in methanol was added and heated on the hot plate.
with the magnetic stirrer set at 50°C for 30 minutes. After the reaction took place, the solids were filtered and the solvent (methanol) was evaporated using the same techniques described during the lipid extraction method in Section 3.9. Sodium sulfate anhydrous was used to remove moisture from the transestrified oil. Evaporation took place until approximately 1 ml of the solution remained. Then, Chloroform was added into the flask to dissolve the produced FAMEs until the total volume of the mixture reached 10 ml. This final product was transferred to a 20 ml glass vial and it was ready for the gas chromatograph (GC) analysis to determine the FAME yield and distribution.

3.10.2 IN-SITU TRANSESTRIFICATION

The two-step process (conventional transestrification) described in Section 3.9 and Section 3.10.1 took place in about 5 hours in practice, which is time consuming. Moreover, it was costly since the solvent used for extracted the lipids (n-hexane) is expensive. On the other hand, one-step process (in-situ transestrification) did not use any hazardous or expensive materials and took only 30 minutes operating time. Therefore, the one-step process (in-situ transestrification) is recommended for biodiesel production. Figure 3.4 shows a schematic of the two-step process (conventional transestrification) versus the one-step process (In-situ transestrification)
**One Step Process** (5 hrs. operating time)

- **Algae Growth & Harvesting**
- **In-Situ Transesterification**
  - Using 0.1 N KOH in Methanol
  - Less time, No Hexane

- **Harvested algae**

**Two Step Process** (30 min operating time)

- **Oil Extraction**
  - Using n-Hexane
- **Trans-esterification**
  - Using 0.1 N KOH in Methanol

**CAUTION**

**HAZARDOUS MATERIAL**

Biodiesel

Figure 3.4: The one-step process for biodiesel production versus the two-step process

W375 ultrasonicator was used for this study. The aim of the sonication was to break the algae cell membrane and release algae oil. The transestrification reaction took place at the same time. 1 g of pulverized algae was added into 150 ml glass beaker. 40 ml of 0.1 KOH in methanol were added to the same beaker. The mixture was sonicated using the W375 ultrasonicator set at a power density of 9.4 W/ml and a pulsed duty cycle of 50%. The effect of the sonication and reaction time was studied during this investigation for optimum FAME yield. After the reaction is completed, the mixture was filtered using 0.25 µm Whatman filter paper grade #5 to remove the remaining algae particles then the mixture was evaporated using the water bath set at 40°C and the supplied air to remove the solvent (methanol). Chloroform was added with the same techniques mentioned in Section 3.10.1 to get the FAMEs ready for the GC analysis to determine the FAME yield. Figure 3.5 show W375 ultrasonicator used for in-situ transestrification.
3.11 GAS CHROMATOGRAPH (GC) PROCEDURE AND DATA ANALYSIS

3.11.1 GC PROCEDURE

Hewlett Packard HP 5890 Series II Gas chromatograph (GC) was used to identify the different biodiesel FAMEs. The GC consists of the following parts:

I. An oven, which contained the column. The Column used was RTX fused silica fast column 30 m long, 5.0 μm film thickness and 0.32 mm inner diameter.

II. A cool on-Column injection port used to inject the FAME sample.

III. A flame ionization detector supplied with Hydrogen gas used to ignite the flame.
3μL of the solution (FAME dissolved in chloroform) was injected into the cool on-column at 50 °C using a 5 μL Hamilton syringe. The total run period was 25 minutes. The GC was connected to an HP integrator used to convert the GC raw data to a chromatogram. The integrator was connected to a computer loaded with HP Peak96 software. Peak96 collected the data and saved it as an ASCII “asc” format. Then, the “.asc” data file was transferred to laptop and converted to comma-separated values (sometimes called character-separated values) “csv” format. The .csv file was opened in Excel for data analysis.

3.11.2 GC METHOD

The GC method is the description of the temperature program at which the column was set at. The GC column was set as follows:

Oven temperature = 240°C

The initial temperature = 240 °C

The final temperature = 275 °C

Initial time = 2.00 min

Final time =10.00 min

Rate = 15 °C/min

Injection temperature = 275°C

Detection temperature = 280°C

Flow rate of Helium = 1.34 ml/min

Helium was used as the carrier gas, flowing at 1.34 ml/min or about 24.5 cm/s at 50 C.
Thus, Oven temperature (or column temperature) profile start at 240 °C and kept for 2 minutes, then ramped to 275°C at 15°C /min. The final temperature was held for 10 minutes.

3.11.3 GC DATA ANALYSIS

Biodiesel FAMEs were identified and quantified by the following steps:

a. Analytical reference standards obtained from Restek were prepared to known concentration as follows:

Each of C18:1, C18:2 and C18:3 were in liquid phase placed in an ampule. Each ampule contained 100 mg of the standard. The top of the ampule was broken down and the liquid was poured in 20 ml glass vial. 10 ml of chloroform were added to the vial to dilute the standard at a concentration of 10 mg/ml. For more dilution, 1 ml of this standard at 10 mg/ml was taken in another glass vial and 5 ml of chloroform were added to dilute the standard at a concentration of 10/6 = 1.67mg/ml.

C16:0 was a frozen solid placed in an ampule. The ampule contained 100 mg of C16:0. The top of the ampule was broken and the entire 100 mg solid was transferred in a 20 ml glass vial. 10 ml of chloroform was used to dissolve the standard and dilute it at a concentration of 10 mg/ml. For more dilution, 1 ml of this standard at 10 mg/ml was taken in another glass vial and 5 ml of chloroform were added to dilute the standard at a concentration of 1.67mg/ml.

C18:0 was a 100 mg powder. 0.012 g = 12 mg was simply weighed using Optima scale and dissolved in 10 ml of chloroform to dilute the C18:0 at a concentration of 1.2 mg/ml.
b. Each standard with known concentration was injected in the GC individually to determine its retention time and the integrated area of the identified peak.

c. Two different cocktails were prepared. Each cocktail contained a mixture of the standards; C16:0, C18:1, C18:2, C18:3 and C18:0. Certain volume of each standard was measured using the digital micro-pipette 20-200 µl and added to the same 2 ml glass vial. The concentration of each standard in the cocktail was calculated. Each cocktail was injected in the GC to identify FAME peaks and confirm retention time.

d. The area (X) under each peak was proportional to the corresponding concentration of the same standard. Therefore, the concentration of each unknown FAME in the biodiesel sample (Y_i) was calculated using the known concentration of that FAME (standard) when injected individually (Y_s) according to the equation 3.3

\[ Y_i = Y_s \frac{X_i}{X_s} \]  

Equation 3.3

where \( Y_i \) and \( X_i \) are the concentration of the unknown FAME and the corresponding area under the peak respectively. \( Y_s \) and \( X_s \) are the concentration of that FAME when injected individually and the corresponding area under the peak respectively.

For example, consider \( X_i \) is the area under the peak of C18:0 in a biodiesel sample equal 256221 and the results showed that \( X_s \) which was the area under C18:0 when injected individually equal 259342 with a corresponding concentration \( Y_s \) 1.2 mg/ml, so the concentration of C18:0 in that biodiesel sample calculated using equation 3.3
\[ Y_i = 1.2 \times \frac{256221}{259342} = 1.18 \text{mg/ml} \]

These calculations were applied to calculate the concentrations of C16:0, C16:1 and C18:0 in the biodiesel sample. C18:1, C18:2 and C18:3 showed as overlapped peak. Another cocktail was prepared of the three standards; C18:1, C18:2 and C18:3 at equal concentration of each of 0.557 mg/ml. The area under the overlapped peak was 667075. The concentration of the three standards; C18:1, C18:2 and C18:3 in the biodiesel sample were calculated using the accumulation of their concentration in the cocktail, which is 1.67 mg/ml with the corresponding area 667075.

The peaks of a biodiesel sample could be identified by comparing their retention time by the retention time of the standards when they were injected individually. Any peak that has a different retention time other than the known standards is considered an unknown peak.

The total FAME yield could be calculated by the sum of the resulted concentration of C18:0, C18:1, C18:2, C18:3, C16:0 and C16:1 multiplied by the total volume of biodiesel and chloroform to determine the total FAME yield per 1 g of dry algae used for in-situ or conventional transestrification.

### 3.12 BLENDING PROCEDURE

Biodiesel from waste vegetable oil was used as a readily-available analogue for algal biodiesel in preliminary blending experiments. Biodiesel from waste vegetable oil (B100) was blended with jet-fuel to produce bio-jet fuel. The maximum ratio of
biodiesel to jet fuel prepared was 50:50 by volume to obtain the specification properties. Bio-jet blends with different ratios; 40 biodiesel: 60 jet fuel, 30:70, 20:80 and 10:90 were prepared to determine their properties: specific gravity, freezing point and heat of combustion.

3.13 BIO-JET BLEND PROPERTIES

3.13.1 BIO-JET BLEND SPECIFIC GRAVITY DETERMINATION

The densities of jet fuel and B100 were easily determined by measuring certain volume of each fuel and weighing their masses. Then the specific gravity of each fuel \( SG_i \) was determined using the density of that fuel with respect to the density of water (1 kg/l or 1 g/ml) using Equation 3.4

\[
SG_i = \frac{\rho_i}{\rho_{\text{water}}}
\]  

Equation 3.4

where \( \rho_i \) and \( \rho_{\text{water}} \) are the density of the fuel \( i \) and the density of water respectively.

Mixing rules were used to estimate the specific gravity of the blend. The specific gravity of the blend with different ratios was calculated from the specific gravities of jet fuel and of B100 using Equation 3.5

\[
SG_{\text{blend}} = \sum \left( \frac{SG_i}{m_i} \right)
\]  

Equation 3.5

where \( SG_{\text{blend}} \) is the specific gravity of the blend, \( m_i \) is the mass fraction of fuel \( i \) in the blend and \( SG_i \) is the specific gravity of fuel \( i \).
Equation 3.5 was also used to determine the specific gravity of vulgaris biodiesel, produced in this study, when it blends with jet fuel. Results will be discussed in Chapter 4, Section 4.9.1

3.13.2 BIO-JET BLEND FREEZING POINT DETERMINATION

The property of freezing point depression was used to create a mixture that has a freezing point at a targeted temperature. This mixture can be used to cool samples to an intermediate temperature that may be between the temperatures obtained with refrigeration. Cryoscopic equation (equation 3.6) was used to calculate the freezing point of a mixture of ethanol and water.

\[ \Delta T_f = T_f(\text{solution}) - T_f(\text{solvent}) = -i \times K_f \times m \]  

Equation 3.6

Where \( T \) values are freezing point for each substance, \( \Delta T_f \) is Difference between freezing point of the mixture and of the pure solvent, \( K_f \) is a cryoscopic constant of the solvent, \( m \) is molality, and \( i \) is the Vant Hoff factor (Engel and Reid, 2004).

Ethanol and water mixtures have well-documented freezing points, so are ideal in many ways for this application. Ethanol was considered as a solvent. Hence, Vant Hoff factor of ethanol = 1, the cryoscopic constant of ethanol = 2 [K/(mol/kg)] and the molality (\( m \)) can be calculated using equation 3.6. Accordingly, the weight percentage of ethanol in the mixture was determined, i.e. 68.9 % weight of methanol in the mixture creates a liquid with the freezing point of -50 °C.

Once the mixture with the desired freezing point (-50 °C) has been created, it is placed in a silicon ice tray and frozen in a freezer with a temperature lower than the
freezing point. This freezer was available at the DRC (-80 °C). When the solution has solidified, it is emptied into an insulated tray in a cool room. No additional solvent is added. The solidified mixture will quickly begin to melt. Submerging the sample jars into the ice bath allows them to be brought to the freezing point temperature. The bath must be carefully stirred with a rod to maintain thermal equilibrium. As long as solid particles remain in the ice bath, it will not have warmed beyond the freezing point temperature. A digital thermometer was used to closely monitor the temperature of the sample and the ice bath. A correlation between the freezing point and the blending ratio was investigated.

3.13.3 BIO-JET BLEND HEAT OF COMBUSTION DETERMINATION

Bomb calorimetry was used for measuring combustion enthalpy of pure jet fuel, B100, and bio-jet blends with different ratios of B100: jet fuel. The correlation between the blend ratio and the heat of combustion was investigated.

Figure 3.6 shows the cross section of a typical adiabatic bomb calorimeter. The combustion reaction is carried out in the bomb, a thick-walled metal container, which is immersed in a water bucket. The energy released by the reaction causes the temperature of the calorimeter to increase. This temperature change is measured with thermometer. The heating value was calculated using Equation 3.7

\[
\frac{Heating\ value\ of\ the\ fuel}{m_{fuel}} = \frac{q_{fuel}}{m_{fuel}} = \frac{-(C_{cal} \Delta T + q_{wire})}{m_{fuel}}
\]

Equation 3.7
Figure 3.6 Cross section of an adiabatic bomb calorimeter (Gonghu, 2013)

where \( q_{\text{fuel}} \) is the heat released by the combustion of the fuel, KJ/g, \( m_{\text{fuel}} \) is the mass of the fuel, g, \( C_{\text{cal}} \) is the heat capacity of the calorimeter, KJ/g, \( \Delta T \) is the temperature change, °C, measured by the thermometer and \( q_{\text{wire}} \) is the heat released by the combustion of the wire, KJ/g.

3.14 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

3.14.1 JMP SOFTWARE

JMP is a statics software program used in different applications such as quality control, engineering, design of experiments and scientific research. JMP is used to analyze the data and links statistical data to graphics representing them. JMP is using in this study to analyze the data.
The goal of our case study is to select the light source and the water source, which result in producing the highest lipid productivity (mg lipids/L Solution-day) in case of each species of algae; Chlorella C2 and Chlorella vulgaris. Two factors experiment was conducted: light source and water source. The experiment was replicated three times. The response is the measured of the lipid production. One way analysis of variance and each pair student's t test were used through JMP software in order to analyze the data.

3.14.2 ONE WAY ANALYSIS OF VARIANCE

Analysis of variance (ANOVA) is the process of subdivision the total variability of experimental observations into portions attributable to recognized sources of variation (Lentner et al., 1993, Ramsey, 2004). Hence, the total variation in the response is separated into two categories: Systematic or large-scale variation cause by our treatments and the experimental error, which has no assignable cause. Experimental error is small-scale variation and is estimated from the replicates. One way ANOVA analysis can be obtained by selecting the menu option Fit Model from Analyze (See Appendix III) Significant conclusions can be provided using the Prediction profile (See Chapter 4, Section 4.11.1)

3.14.3 EACH PAIR STUDENT'S T TEST

Each Pair Student's t Test is the procedure that compares each pair of the parameters of each factor. It compares all the possible pairs. It has the highest power of
the mean separation procedures such as Hsu's Multiple Comparison with the best, Dunnett's Test for treatment levels vs. a control and Tukey-Kramer Honestly Significant Difference, Ramsey, 2004. Student's t test comparison can be obtained by selecting the menu option Fit Y by X from Analyze (see Appendix III for the detailed steps). The Comparison Circles can be used to determine which treatments form a class of best treatment. The results were interpreted by clicking on the Comparison Circles or reviewing the test report, See Chapter 4, section 4.11.2.

3.14.4 DESIGN OF EXPERIMENTS OVERVIEW AND EXPERIMENTAL PLAN

When experimenting with algae growth there were
- Two strains of Chlorella (V = vulgaris and C2)
- Two sources of water (WF = Fresh and WW = wastewater) (sample size is 2L)
- Three sources of light (F = Fluorescent, RL = red LEDs, RBL = red-blue LEDs)

Experiments were designed to obtain data for each of the following cases
1- V WF F
2- V WF RL
3- V WF RBL
4- V WW F
5- V WW RL
6- V WW RBL

Cases 7 - 12 are the same as 1-6 except using C2, i.e., 7- C2 WF F etc.
Each experiment was replicated three times. Hence, there were a total of 36 runs for algae growth. As shown in Chapter 4, Section 4.11 the data was entered into the JMP software for statistical data analysis.
CHAPTER IV

4 RESULTS AND DISCUSSION

The objectives of the present work, as stated in Chapter 1, are summarized in Table 4.1

Table 4.1: Project objectives and sections in which the objective is discussed

<table>
<thead>
<tr>
<th>No.</th>
<th>Objective Description</th>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Investigate minimizing the energy requirements for microalgal growth by replacing</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>fluorescent lights with light emitting diodes (LEDs).</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Investigate the use of municipal waste water in growing microalgae to reduce fresh</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>water use.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Study the effect of light intensity on algae oil production.</td>
<td>4.4</td>
</tr>
<tr>
<td>4</td>
<td>Kinetic study of microalgal growth</td>
<td>4.5</td>
</tr>
<tr>
<td>5</td>
<td>Investigate the scale up of microalgal growth in fresh water and waste water from</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>2 Liters to 80 Liters using fluorescent light and red-blue LEDs.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Determine the Fatty Acid Methyl Ester (FAME) composition of the microalgae biodiesel</td>
<td>4.7&amp;</td>
</tr>
<tr>
<td></td>
<td>and use it to predict its physical properties.</td>
<td>4.8</td>
</tr>
<tr>
<td>7</td>
<td>Study the one-step production of biodiesel using in situ algal biomass transestrif</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>ication process to reduce production time and cost.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Study the nitrogen starvation effect on the fatty acid methyl esters (FAMEs)</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>concentration.</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Investigate biodiesel blending with jet-fuel in order to obtain bio-jet fuel.</td>
<td>4.9</td>
</tr>
<tr>
<td>10</td>
<td>Determine the properties of the blend of different biodiesel: jet-fuel ratios. The</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>properties include specific gravity, freezing points, and heat of combustion.</td>
<td></td>
</tr>
</tbody>
</table>

These objectives were based on the project hypotheses summarized in Table 4.2
Table 4.2: Project hypotheses

<table>
<thead>
<tr>
<th>No</th>
<th>Hypothesis Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LEDs can be effectively used to grow algae with comparable or better algae lipid production using fluorescent lights, making the process greener and more energy efficient.</td>
</tr>
<tr>
<td>2</td>
<td>Lipid-containing microalgae species can be grown using waste water effluent from a municipal waste water treatment plant. In addition, the algae production and lipids productivity in municipal waste water will be comparable to algae growth in fresh water using the same nutrients, reducing the fresh water footprint.</td>
</tr>
<tr>
<td>3</td>
<td>Higher incident light intensity increases the production of algae biomass and lipids.</td>
</tr>
<tr>
<td>4</td>
<td>Scale-up of microalgae growth PBR will reduce algae biomass production and lipid production</td>
</tr>
<tr>
<td>5</td>
<td>Physical properties of microalgae biodiesel can be predicted from the Fatty Acid composition.</td>
</tr>
<tr>
<td>6</td>
<td>One step process avoids using any hazardous materials such as hexane and saves time. Thus, in-situ transestrification improves the biodiesel production time and economics.</td>
</tr>
<tr>
<td>7</td>
<td>Microalgae lipid triggering by nitrogen deprivation will increase the algae lipid content.</td>
</tr>
<tr>
<td>8</td>
<td>Microalgae biodiesel can be blended with petroleum based jet fuel. The resulting liquid fuel will have properties similar to jet fuel, but is a greener drop-in aviation fuel.</td>
</tr>
</tbody>
</table>

The results presented and discussed in this Chapter represent responses to the 10 objectives addressed in Chapter 1, Section 1.15.

4.1 EFFECT OF MEDIUM AND LIGHT SOURCES ON ALGAE GROWTH AND LIPID PRODUCTION (Objectives 1 and 2)

Six of the 2L PBRs were operated concurrently as stated in Chapter 3, Section 3.7.1. The same nutrients were added to both fresh water and waste water mediums. The light
intensity of the different light sources was maintained at 2000 LUX. The growth conditions of the 6 PBRs (6 runs) are listed in Table 4.3

Table 4.3: Operating conditions of six of the 2L PBRs

<table>
<thead>
<tr>
<th>PBR</th>
<th>Medium Source</th>
<th>Light Source</th>
<th>Light Intensity, LUX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fresh water</td>
<td>Red LEDs</td>
<td>2000</td>
</tr>
<tr>
<td>2</td>
<td>Waste water</td>
<td>Red LEDs</td>
<td>2000</td>
</tr>
<tr>
<td>3</td>
<td>Fresh water</td>
<td>Red-Blue LEDs</td>
<td>2000</td>
</tr>
<tr>
<td>4</td>
<td>Waste water</td>
<td>Red-Blue LEDs</td>
<td>2000</td>
</tr>
<tr>
<td>5</td>
<td>Fresh water</td>
<td>Fluorescent</td>
<td>2000</td>
</tr>
<tr>
<td>6</td>
<td>Waste water</td>
<td>Fluorescent</td>
<td>2000</td>
</tr>
</tbody>
</table>

PBRs (1 and 2), (3 and 4) and (5 and 6) compare waste water to fresh water. Also, PBRs (1, 3, 5) and PBRs (2, 4, 6) compare the effect of light source on algae growth and lipid production. Hence these experiments were intended to prove the first two hypotheses of this project listed in Table 4.2. The total energy saving using LEDs relative to fluorescent light is about 48.5% (68 W relative to 35 W). The saving is comparable with that of Chen et al., 2011 (See Chapter 2, section 2.3.2). These experiments were run using Chlorella vulgaris and Chlorella C2.

4.1.1 TRANSIENT EFFECT OF MEDIUM AND LIGHT SOURCES ON ALGAE GROWTH

Daily turbidity and cell count measurements of the 6 PBRs were taken every other day in order to monitor and observe algae growth. Turbidity of the algae solution is proportional to algae concentration in the growth medium. Hence, higher turbidity of the
algae solution indicates higher growth rate at the same algae culture growth period. These experiments were run using two species; Chlorella vulgaris and Chlorella salina (Chlorella C2). The growth period of Chlorella vulgaris and Chlorella C2 were 18 and 14 days respectively.

Figures 4.1 (a) and (b) show the turbidity and cell count results of Chlorella vulgaris respectively.

![Figure 4.1 (a): Effect of growth medium and 2000 LUX light sources on the transient Chlorella vulgaris turbidity at room temperature.](image)

The turbidity results of Chlorella vulgaris showed that Fresh water, Red LEDs run reached the highest turbidity (2.56 arbitrary unit) at the end of the 18 days growth period followed by the Waste water, Red-Blue LEDs (1.44 arbitrary unit), while the lowest turbidity were obtained by the Fresh water, Fluorescent (0.46 arbitrary unit) and the Waste water Fluorescent (0.6 arbitrary unit) runs.
Figure 4.1 (b): Effect of growth medium and 2000 LUX light sources on the transient Chlorella vulgaris cell concentration at room temperature.

The cell count results of Chlorella vulgaris showed that Waste water, Red-Blue LEDs run reached the highest cell count (15.4 million cells/ml) at the end of the 18 days growth period followed by Fresh Water, Red LEDs run (13.4 million cells/ml). Hence, they showed the highest growth rates. On the other hand, the lowest cell counts were obtained by the Fresh water, Fluorescent and the Waste water, Fluorescent runs. It is important to note that the cell counts results were in agreement with the turbidity results. Both show that:

- Wastewater is a promising replacement for fresh water in algae growth (Hypothesis 1).
- LEDs are better light source for algae growth than fluorescent at the same light intensity (Hypothesis 2).

Figures 4.2 (a) and (b) show the turbidity and cell count results of Chlorella C2 respectively.
Figure 4.2 (a): Effect of growth medium and 2000 LUX light sources on the transient Chlorella C2 turbidity at room temperature.

Figure 4.2 (b): Effect of growth medium and 2000 LUX light sources on the transient Chlorella C2 Cell concentration at room temperature.

It is desired to stop each run as it reaches the beginning of the stationary phase. It is possible to predict when the stationary phase will begin by observing growth during the lag and exponential phases. Figures 4.2 (a) and (b) show that Fresh water, Fluorescent
and Waste water, Fluorescent runs had a long lag period of 8 days and exponential phase of 6 days and entered the stationary phase at day 14. Therefore, these two runs were stopped at day 14 before the cells enter the lysis phase. Fresh water, Red LEDs and Waste water, Red LEDs runs had a short lag phase of 5 days and long exponential phase of 9 days. Then, these runs were stopped when they reached high cell concentration.

Chlorella vulgaris, Figures 4.1 (a) and (b), and Chlorella C2, Figures 4.2 (a) and (b), show the same effect of growth medium and light source on turbidity and cell counts. All figures confirm the validity of Hypothesis 1 and 2.

4.1.2 TRANSIENT EFFECT OF MEDIUM AND LIGHT SOURCES ON PH

Measurements of algae growth medium pH were taken by Mardel strips, as indicated in Chapter 3, Section 3.7.1, in order to show the acidity of the solution and monitor the algae growth. Hence, Algae culture should be harvested when the pH measurements were maintained at which it reached the stationary phase.

The pH started at 6.8 and increased until it reached 8.4 at the end of the growth period for the different runs at different light sources and medium sources as shown in Figure 4.3
Figure 4.3: Effect of growth medium and 2000 LUX light sources on the transient pH of Chlorella vulgaris at room temperature.

4.1.3 Nitrate, Nitrite

Nitrate and Nitrite concentrations were measured during the growth period of each run. The data are intended to confirm that the growth medium contained enough nitrates and nitrites. Hence the algae growth continued until reaching the stationary phase without nitrogen starvation. These measurements were taken using Mardel strips (see Chapter 3, Section 3.7.1).

Table 4.4 (a) and (b) list the nitrate and nitrite measurements respectively during the growth period.
Table 4.4 (a): Effect of growth medium and 2000 LUX light sources on the transient nitrate concentration at room temperature.

<table>
<thead>
<tr>
<th>Day</th>
<th>Fresh water, Red LEDs</th>
<th>Waste water, Red LEDs</th>
<th>Fresh water, Red-Blue LEDs</th>
<th>Waste water, Red-Blue LEDs</th>
<th>Fresh water, Fluorescent</th>
<th>Waste water Fluorescent</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>7</td>
<td>160</td>
<td>200</td>
<td>160</td>
<td>160-200</td>
<td>160</td>
<td>160-200</td>
</tr>
<tr>
<td>11</td>
<td>160</td>
<td>200</td>
<td>80</td>
<td>80</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>13</td>
<td>80</td>
<td>80-160</td>
<td>160-80</td>
<td>80-160</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>14</td>
<td>80</td>
<td>80</td>
<td>160</td>
<td>80</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

Table 4.4 (b): Effect of growth medium and 2000 LUX light sources on the transient nitrate concentration at room temperature.

<table>
<thead>
<tr>
<th>Day</th>
<th>Fresh water, Red LEDs</th>
<th>Waste water, Red LEDs</th>
<th>Fresh water, Red-Blue LEDs</th>
<th>Waste water, Red-Blue LEDs</th>
<th>Fresh water, Fluorescent</th>
<th>Waste water Fluorescent</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.5-1</td>
<td>5-10</td>
<td>0.5</td>
<td>5-10</td>
<td>0-0.5</td>
<td>3-5</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>0.5</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>5-10</td>
<td>0.5</td>
<td>3-5</td>
<td>1</td>
<td>5-10</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>10</td>
<td>3</td>
<td>3-5</td>
<td>1-3</td>
<td>5-10</td>
</tr>
</tbody>
</table>

The data confirm the presence of nitrate and nitrate during the algae growth.

4.1.4 EFFECT OF MEDIUM AND LIGHT SOURCES ON ALGAE BIOMASS PRODUCTION

After algae harvested, centrifuged and freeze dried, the mass of the freeze dried algae was weighed and algae biomass production were calculated using equation 3.1. For example, the 2L Fresh water, Red LEDs run provided 2.24 g (= 2240 mg) of dry algae,
the volume of the harvested algae solution was 1.15 L. The growth period of this run was 18 days. The daily algae biomass production for this run can be calculated as follows:

**Algae biomass production, g/L Solution-day**

\[
\text{Algae biomass production} = \frac{2240 \text{ mg}}{(1.15 \text{ L}) \times (18 \text{ day})} = 108.2 \text{ mg/L Solution-day}
\]

![Figure 4.4: Effect of growth medium and 2000 LUX light sources on Chlorella vulgaris biomass production at room temperature.](image)

According to Figure 4.4 the highest Chlorella vulgaris biomass production (177.8 mg dry algae/L Solution-day) was obtained using Fresh water medium and Red-Blue LEDs. The light source had slight effect on Chlorella vulgaris biomass production in waste water medium runs. Chlorella vulgaris biomass production in waste water are 64%, 51% and 76% of that of Fresh water using Red LEDs, Red-Blue LEDs and fluorescent light respectively.
Figure 4.5 shows the effect of growth medium and light sources on Chlorella C2 biomass production. Red LEDs are the most effective light source resulting in the highest biomass production in both fresh water and waste water. Chlorella C2 biomass production in waste water are 69%, 104% and 24% of that in fresh water using Red LEDs, Red-Blue LEDs and fluorescent light respectively.

Figure 4.5: Effect of growth medium and 2000 LUX light sources on Chlorella C2 biomass production at room temperature.

These experiments were replicated three times for each species; Chlorella vulgaris and Chlorella C2. The biomass production results of the replicates are shown in Appendix IV.
4.1.5 EFFECT OF MEDIUM AND LIGHT SOURCES ON ALGAE LIPID PRODUCTION

Figure 4.6 shows the effect of medium and the light source on algae lipid yield (g lipid/100 g dry algae). The highest lipid yield was obtained using Fresh water medium and Red LEDs. However, high lipid content is often obtained at the expense of the algae biomass production. Thus, algae lipid content is not the best measure of the algae lipids/oil production. Algae lipid production, which combine the effect of algae lipid content and algae biomass production should be a better indicator. Therefore, the comparisons of this study are based on the results of the oil productivity in mg lipid/L solution-day. Algae lipid production was calculated using equation 4.1

\[
\text{Algae lipid production, mg lipids/L-day} = \\
\text{Algae biomass production, mg dry algae/L-day} \times \text{Lipid Yield, g lipids/100 g dry algae}
\]

Equation 4.1
Figure 4.6: Effect of growth medium and 2000 LUX light sources on the Chlorella vulgaris lipid yield at room temperature.

The results of Chlorella vulgaris lipid production are shown in Figure 4.7.

Figure 4.7: Effect of growth medium and 2000 LUX light sources on the Chlorella vulgaris lipid production at room temperature.
The use of Fresh water growth medium and Red-Blue LEDs resulted in the highest Chlorella vulgaris lipid production (12.45 mg lipid/L Solution-day). Figure 4.7 shows that Chlorella vulgaris lipid production in waste water was slightly affected by the light source, representing about 30% of the highest lipid production obtained by Fresh water, Red-Blue LEDs.

As discussed in Chapter 1, Section 1.8.1 Mallick et al. (2012) studied the growth cycle and lipid accumulation. Table 4.5 compares the present work results with Mallick et al., 2012 and Gouveia et al., 2009.

Table 4.5: Comparison of chlorella vulgaris biomass production and lipid yield of present work, Gouveia et al., 2009 and Mallick et al., 2012.

<table>
<thead>
<tr>
<th>Chlorella vulgaris</th>
<th>Present work (Fresh water, Red-Blue LEDs)</th>
<th>Gouveia et al., 2009</th>
<th>Mallick et al., 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass production, mg dry algae/L Solution-day</td>
<td>177.8</td>
<td>180</td>
<td>43.63</td>
</tr>
<tr>
<td>Lipid yield, % dry wt.</td>
<td>7</td>
<td>5.1</td>
<td>9.2</td>
</tr>
<tr>
<td>Lipid production, mg lipid/L Solution-day</td>
<td>12.45</td>
<td>9.18</td>
<td>4.01</td>
</tr>
</tbody>
</table>

Figure 4.8 shows the effect of medium and light source on Chlorella C2 lipid yield. It indicates that higher lipids can be obtained using LEDs in both fresh water and waste water than using Fluorescent light. The effect of light and growth medium sources on Chlorella C2 lipid production is shown in Figure 4.9. It shows that the lipid production is almost unaffected by the growth medium and is mainly dependent on the light source. The highest lipid production was obtained using red LEDs.
Figure 4.8: Effect of growth medium and 2000 LUX light sources on Chlorella C2 lipid yield at room temperature.

Figure 4.9: Effect of growth medium and 2000 LUX light sources on Chlorella C2 lipid production at room temperature.

The lipid yield and production results of the replicates for Chlorella vulgaris and Chlorella C2 are listed in Appendix IV.
4.2 LIGHT CAPTURE EFFICIENCY (PHOTOSYNTHETIC EFFICIENCY)

The Photosynthetic efficiency or light capture efficiency is the percent of the light energy transferred through PBR and converted to biomass. It is the ratio of the energy produced by the combustion of the algae biomass product to the incident light energy produced by the light source used. This calculation was based on the measured microalgae heating value of 17.44 KJ/g = 7498 Btu/lb. Figure 4.10 show that the light capture efficiency of Chlorella vulgaris was between 3.6% (Waste water, Red LEDs) and 8.4% (Fresh water, Red-Blue LEDs). For waste water and Red-Blue LEDs run, the 4.7% light capture efficiency, is about 56% of the maximum value of 8.4% of the Fresh water, Red-Blue LEDs run.

Figure 4.10: Effect of growth medium and 2000 LUX light sources on Chlorella vulgaris light capture efficiency at the end of 18 days growth period at room temperature.

Figure 4.11 shows that the highest light capture efficiency of 12.9 % was obtained by Chlorella C2 growing in Fresh water using Red LEDs. Moreover, the light capture efficiency of Waste water, Red LEDs run (8.9%), and Waste water Red LEDs (4.6%)
were 69 and 36% of the highest light capture efficiency of 12.9% of Fresh water, Red LEDs respectively. The fresh water fluorescent light run had a light capture efficiency of about 3.1%. This compares well with the 2.53% efficiency of Zemke, et al. (2008) microalgae pond bioreactor.

Figure 4.11: Effect of growth medium and 2000 LUX light sources on Chlorella C2 light capture efficiency at the end of 14 days growth period at room temperature.

4.3 CARBON CAPTURE EFFICIENCY (CARBON SEQUESTERING EFFICIENCY)

Microalgae have higher photosynthetic efficiency than plants. Therefore, they are more efficient in capturing carbon. Microalgae absorb CO2, which stimulate their growth and produce oxygen in the photosynthesis process (See Reaction 1.2). Algae carbon capture efficiency depends on the algae species (Malinska et al., 2010). Carbon capture efficiency or carbon sequestering efficiency is the mass of C sequestered by the algae relative to the mass of C supplied to the PBR. It was calculated by determining the ratio
of the carbon in the dry algae formed to the total carbon from the air into the PBR (Wilson et al., 2012 and Elmoraghy et al., 2012).

The following assumptions were made for this calculation:

- Constant (average) air flow rate into the PBR.
- CO₂ in air (394 ppmv) is the only carbon source for algae.
- Based on literature survey, dry algae contain 45% Carbon
- 44 g CO₂ contain 12 g C.

Figure 4.12: Effect of growth medium and 2000 LUX light sources on Chlorella vulgaris carbon capture efficiency at the end of 18 days growth period at room temperature.
Figures 4.12 show that the carbon sequestration efficiency of Chlorella vulgaris was between 3.4% (Waste water, Fluorescent lights) and 8.6% (Fresh water, Red -Blue). However, the highest carbon capture efficiency of 13.15% was obtained by growing Chlorella C2 in Fresh water using Red LEDs as shown in Figure 4.13. These values were close to the 12% efficiency of McConnell et al., 2012, but higher than that obtained by Wilson et al., 2012. Their results of the carbon sequestration efficiency of microalgae was between 3.4-8.6%

4.4 LIGHT INTENSITY (Objective 3)

The effect of light intensity on the growth of algae and the production of oil/lipid were studied using Fluorescent light, Red LEDs and Red-Blue LEDs. These experiments were intended to prove the third hypotheses of this project listed in Table 4.2.
4.4.1 EFFECT OF FLUORESCENT LIGHT INTENSITY ON ALGAE BIOMASS AND LIPID PRODUCTION

For this study, two different fluorescent light intensities were selected; 2000 LUX (27μmol/m^2.s) and 8000 LUX (108μmol/m^2.s). Two fish tanks were operated concurrently; the first fish tank was illuminated with fluorescent light at 2000 LUX (27μmol/m^2.s) and the second at 8000 LUX (108μmol/m^2.s). Each fish tank was divided into two identical PBRs. The first PBR contained Fresh water medium and the second had Waste water medium. Figure 4.14 shows the resulting effect of fluorescent light intensity on Chlorella vulgaris algae growth in fresh water and in waste water. The result shows that Chlorella vulgaris biomass production almost doubles with the four times increase in light intensity for both medium, i.e., the production rate is dependent on the square root of the light intensity. This is consistent with the observation in the ultraviolet (UV) curing of coatings, varnishes and adhesives. A presentation by Cork Industries, located at 500 Kaiser Drive Folcroft, PA 19032 phone: 610.522.9550 (http://www.corkind.com/catalogs/UV_Book.pdf) states; “UV light intensity directly affects cure rate, which changes as the square root of the light intensity increases.”
Figure 4.14: Effect of Fluorescent light intensity on Chlorella vulgaris biomass production in fresh water and waste water at the end of 15 and 18 days algae growth periods at room temperature.

Figure 4.15: Effect of Fluorescent light intensity on Chlorella vulgaris lipid production in fresh water and waste water at the end of 15 and 18 days algae growth periods at room temperature.
Figure 4.15 shows the effect of Fluorescent light intensity on Chlorella vulgaris lipid production in fresh water and in waste water growth medium. Both fresh water and waste water show increase in Chlorella vulgaris lipid production. However, Chlorella vulgaris lipid production improvement in waste water from 1.34 to 5.13 mg lipid/L Solution-day is 33% of that improvement in fresh water from 0.88 to 8.16 mg/L Solution-day.

The Fluorescent light intensity effect in case of Chlorella C2 indicates that waste water is promising as the improvement of Chlorella C2 biomass production in waste water is relatively double their improvement in fresh water. Moreover, the improvement of Chlorella C2 lipid production in waste water is 10% more than the improvement in fresh water. See Figures 4.16 and 4.17.

Figure 4.16: Effect of Fluorescent light intensity on Chlorella C2 biomass production in fresh water and waste water at the end of 14 and 13 days algae growth period at room temperature.
Figure 4.17: Effect of Fluorescent light intensity on Chlorella C2 lipid production in fresh water and waste water at the end of 14 and 13 days algae growth period at room temperature.

4.4.2 EFFECT OF LEDS LIGHT INTENSITY ON ALGAE BIOMASS AND LIPID PRODUCTION

The most efficient light source in case of Chlorella vulgaris species was Red-Blue LEDs (see Figures 4.4 and 4.7). Therefore, Chlorella vulgaris algae species were selected to study the effect of Red-Blue LEDs light intensity on algae biomass and lipid production. For Chlorella C2 species the most efficient light source in case of was Red LEDs (see Figures 4.5 and 4.9). Therefore, we choose Chlorella C2 algae species to study the effect of Red LEDs light intensity on algae biomass and lipid production. The selections are summarized in Table 4.6.
Table 4.6: Selection of algae and LEDs to study effect of led intensity on algae growth and lipid production

<table>
<thead>
<tr>
<th>Microalgae</th>
<th>LEDs selected</th>
<th>Reason Supporting Figures</th>
<th>Light Intensities used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorella vulgaris</td>
<td>Red-Blue</td>
<td>Highest algae biomass production and highest lipid production.</td>
<td>Figures 4.4 and 4.7.</td>
</tr>
<tr>
<td>Chlorella C2</td>
<td>Red</td>
<td>Highest algae biomass production and highest lipid production.</td>
<td>Figures 4.5 and 4.9.</td>
</tr>
</tbody>
</table>

Red-Blue LEDs panels used for this experiment were available in two different light intensities; 2000 LUX and 5500 LUX. Red LEDs panels were available in 1800 LUX and 3500 LUX. Comparisons of different light intensities and their effect were studied in fresh water and waste water.

Figures 4.18 and 4.19 show that the Red-Blue LEDs light intensity effect on Chlorella vulgaris biomass and lipid production in fresh water and waste water. As the light intensity almost doubles from 1800 LUX to 3500 LUX, Chlorella vulgaris biomass production almost doubles in both growth media. Fresh water media increases from 109.1 to 197.2 mg dry algae/L Solution-day. Waste water media increases from 86.2 to 149.4 mg dry algae/L Solution-day. The lipid production increase four times in fresh water (from 1.1 to 4.4 mg lipids/L Solution-day) and five times in waste water (from 0.5 to 2.5 mg lipids/L Solution-day). Chlorella vulgaris biomass production improvement in waste water is 72% of that improvement in fresh water. Moreover, Chlorella vulgaris lipid production improvement in waste water is 60% of that improvement in fresh water.
Figure 4.18: Effect of Red-Blue LEDs light intensity on Chlorella vulgaris biomass production in fresh water and waste water at the end of 13 and 14 days algae growth period at room temperature.

Figure 4.19: Effect of Red-Blue LEDs light intensity on Chlorella vulgaris lipid production in fresh water and waste water at the end of 13 and 14 days algae growth period at room temperature.
Figures 4.20 and 4.21 show the Red LEDs light intensity effect on Chlorella C2 biomass and lipid production in fresh water and waste water. Increasing the light intensity from 2000 LUX to 5500 LUX resulted in increasing both the biomass production and the lipid production. The improvement in waste water is 67% and 69% more than the improvement in fresh water for biomass production and lipid production respectively.

Figure 4.20: Effect of Red LEDs light intensity on Chlorella C2 biomass production in fresh water and waste water at the end of 13 days growth period at room temperature.
Figure 4.21: Effect of Red LEDs light intensity on Chlorella C2 lipid production in fresh water and waste water at the end of 13 days growth period at room temperature.

4.5 KINETIC MODEL OF MICROALGAE GROWTH (Objective 4)

Huesemann, 2009 kinetics model for the determination of the maximum microalgae specific growth rate ($\mu_{\text{max}}$) in batch culture was adopted in the present work. Similar kinetics model was developed by Borja, 2008. The maximum specific growth rate is only determined during the exponential growth period and is calculated by the following equation as.

$$\mu_{\text{max}} = \frac{1}{\Delta t} \ln \left( \frac{C_f}{C_i} \right) \quad \text{Equation 2.6}$$

Where $C_i$ and $C_f$ are the initial and final microalgae biomass concentration in mg/l, respectively, and $\Delta t$ is the length of the exponential phase incubation period in days.
In the present work the final concentration of microalgae is replaced by the microalgae solution turbidity, $T$ of the algae solution at a time $t$, and $\Delta t$ is replaced by $t$, the elapsed time (in days) since the start of the exponential growth started. Eq. 2.6 is written as

$$\mu_{\text{max}} = \frac{1}{t} \ln \left( \frac{T}{T_i} \right)$$

Equation 4.2

This equation is rewritten as

$$\ln T = \mu_{\text{max}} t + \ln (T_i) \quad \text{or} \quad y = \mu_{\text{max}} t + y_i$$

A semi-log plot of logarithm the turbidity, $y = \ln T$, versus the elapsed time $t$ in the exponential growth phase should result in a straight line. The slope of the line is the specific growth rate and the intercept, $y_i = \ln (T_i)$ is the natural log of the turbidity at the start of the exponential phase, when $t = 0$.

Turbidity-time data of Chlorella vulgaris, Figures 4.1(a) and of Chlorella C2, Figure 4.2(a) were used to construct the semi-log plots of Figure 4.21 for Chlorella vulgaris and Figure 4.22 for Chlorella C2. A linear least square fit was done to each of the six data sets.
Figure 4.22: Determination of kinetics parameter of Chlorella vulgaris growth in different media and different light sources

Figure 4.23: Determination of kinetics parameter of Chlorella C2 growth in different media and different light sources
Table 4.7 gives a summary of the kinetics parameters for the six different Chlorella vulgaris growth cases in Figure 4.21. Similarly, Table 4.8 gives a summary of the kinetics parameters for the six different Chlorella C2 growth cases in Figure 4.21.

### Table 4.7: Summary of kinetic parameters of Chlorella vulgaris growth runs at 2000 LUX and at room temperature

<table>
<thead>
<tr>
<th>Medium Source</th>
<th>Light Source</th>
<th>( t_i ) days</th>
<th>( \mu_{\text{max}} \text{ day}^{-1} )</th>
<th>( T_i )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh water</td>
<td>Red LEDs</td>
<td>6</td>
<td>0.1139</td>
<td>0.6</td>
<td>0.9491</td>
</tr>
<tr>
<td>Waste water</td>
<td>Red LEDs</td>
<td>11</td>
<td>0.1538</td>
<td>0.21</td>
<td>0.935</td>
</tr>
<tr>
<td>Fresh water</td>
<td>Red-Blue LEDs</td>
<td>8</td>
<td>0.1404</td>
<td>0.25</td>
<td>0.8538</td>
</tr>
<tr>
<td>Waste water</td>
<td>Red-Blue LEDs</td>
<td>8</td>
<td>0.1573</td>
<td>0.29</td>
<td>0.9735</td>
</tr>
<tr>
<td>Fresh water</td>
<td>Fluorescent</td>
<td>11</td>
<td>0.281</td>
<td>0.07</td>
<td>0.9582</td>
</tr>
<tr>
<td>Waste water</td>
<td>Fluorescent</td>
<td>14</td>
<td>0.2625</td>
<td>0.21</td>
<td>0.8757</td>
</tr>
</tbody>
</table>

\( t_i = \) Starting time of exponential phase, days

\( T_i = \) Initial Turbidity at the start of the exponential phase.

\( R^2 = 1 \) perfect least square fit

### Table 4.8: Summary of kinetic parameters of Chlorella C2 growth runs at 2000 LUX and at room temperature

<table>
<thead>
<tr>
<th>Medium Source</th>
<th>Light Source</th>
<th>( t_i ) days</th>
<th>( \mu_{\text{max}} \text{ day}^{-1} )</th>
<th>( T_i )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh water</td>
<td>Red LEDs</td>
<td>8</td>
<td>0.0893</td>
<td>0.6</td>
<td>0.9979</td>
</tr>
<tr>
<td>Waste water</td>
<td>Red LEDs</td>
<td>10</td>
<td>0.4865</td>
<td>0.04</td>
<td>0.8581</td>
</tr>
<tr>
<td>Fresh water</td>
<td>Red-Blue LEDs</td>
<td>10</td>
<td>0.1237</td>
<td>0.25</td>
<td>0.6266</td>
</tr>
<tr>
<td>Waste water</td>
<td>Red-Blue LEDs</td>
<td>8</td>
<td>0.3015</td>
<td>0.14</td>
<td>0.9723</td>
</tr>
<tr>
<td>Fresh water</td>
<td>Fluorescent</td>
<td>10</td>
<td>0.2882</td>
<td>0.06</td>
<td>0.8483</td>
</tr>
<tr>
<td>Waste water</td>
<td>Fluorescent</td>
<td>10</td>
<td>0.3132</td>
<td>0.06</td>
<td>0.6428</td>
</tr>
</tbody>
</table>

\( t_i = \) Starting time of exponential phase, days

\( T_i = \) Initial Turbidity at the start of the exponential phase.

\( R^2 = 1 \) perfect least square fit
Figure 4.24: Effect of growth medium and 2000 LUX light sources on specific growth rate of Chlorella vulgaris

Figure 4.25: Effect of growth medium and 2000 LUX light sources on specific growth rate of Chlorella C2
Figure 4.24 indicates that $\mu_{\text{max}}$ (day$^{-1}$) of Chlorella vulgaris growth in fresh and waste water are very close in Red LEDs (0.1139 vs. 0.1538), Red-Blue LEDs (0.1404 vs. 0.1573) and fluorescent (0.281 vs. 0.2625). This confirms that waste water is a viable option to replace fresh water. The specific growth rates of fluorescent light in both fresh water and waste water were higher than the others of LEDs. However, the starting point of the exponential phase for LEDs had higher turbidities than the starting points of the fluorescent. This leads us to conclude that either the exponential phase of all runs should start at the same initial turbidity or that the algae growth kinetics during the lag phase is of importance. During the lag period, there is a slight increase in cell mass and volume but no increase in cell number. Hence, the value of specific growth rate alone is not enough to reach a conclusion about the algae growth.

Figure 4.25 indicates that specific growth rate of Chlorella C2 in waste water is even higher than specific growth rate of fresh water in Red LEDs (0.4865 vs. 0.0893), Red-Blue LEDs (0.3015 vs. 0.1237) and fluorescent (0.3132 vs. 0.2882). It is clearly observed that fresh water and red LEDs case show the lowest specific growth rate (0.0893 day$^{-1}$) and the highest turbidity (0.6, Table 4.7) of starting point of the exponential phase, which confirm the previous conclusion.

4.6 SCALE-UP EFFECTS IN COLUMN PBRs (Objective 5)

4.6.1 IMPORTANCE

The design and scale-up of photobioreactors (PBRs) require consideration of the following issues (Weissman et al., 1988):

1- Effective use of incident light;
2- Utilize carbon dioxide (from air) for photosynthesis while minimizing losses;

3- Removal of oxygen generated in the photosynthesis process, otherwise it may inhibit algae metabolism or damage the algae culture.

Algae production in a PBR is strongly dependent upon the light energy that reaches the algae cells. Upon growth the microalgae culture becomes dense (or dark). This decreases the light availability and the photosynthesis. This light decrease by dense algae is termed self-shading (Erickson and Lee, 1986). Self-shading is expected to become more important in the scaling up of column PBRs. It may cause a scaled-up PBR to have lower algae and lipid production than a smaller diameter column PBR.

4.6.2 EFFECT OF MEDIUM SOURCE ON ALGAE GROWTH, AND LIPID CONTENT IN SCALED-UP COLUMN PBR.

Six batches were operated in the 80 L/column PBR (See section 3.7.2) in order to study algae growth in waste water in large scale and compare it with fresh water. The experiments were also intended to validate the fourth hypothesis. Fluorescent light at 14000 LUX was used to illuminate the PBR. However, two trials was operated using the Red-Blue LEDs jacket at 3500 LUX in order to scale up the LEDs usage. The same nutrients were added to each batch with the same concentration used for the 2L PBR (Nutrients were listed in Table 3.4). Table 4.9 lists the labeling of the six batches.
Table 4.9: Labeling of the six batches of the runs in 80 L PBR growth runs at room temperature

<table>
<thead>
<tr>
<th>Label</th>
<th>Algae used</th>
<th>Medium Source</th>
<th>Light Source</th>
<th>Light Intensity, LUX</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Chlorella vulgaris</td>
<td>Fresh water</td>
<td>Fluorescent</td>
<td>14,000</td>
</tr>
<tr>
<td>B</td>
<td>Chlorella vulgaris</td>
<td>Waste water</td>
<td>Fluorescent</td>
<td>14,000</td>
</tr>
<tr>
<td>C</td>
<td>Chlorella C2</td>
<td>Fresh water</td>
<td>Fluorescent</td>
<td>14,000</td>
</tr>
<tr>
<td>D</td>
<td>Chlorella C2</td>
<td>Waste water</td>
<td>Fluorescent</td>
<td>14,000</td>
</tr>
<tr>
<td>E</td>
<td>Chlorella vulgaris</td>
<td>Fresh water</td>
<td>Red-Blue LEDs</td>
<td>3,500</td>
</tr>
<tr>
<td>F</td>
<td>Chlorella vulgaris</td>
<td>Waste water</td>
<td>Red-Blue LEDs</td>
<td>3,500</td>
</tr>
</tbody>
</table>

The results of the biomass production and the lipid yield are summarized in Table 4.10

Moreover, the replication results of the lipid yield are listed in Appendix IV.

Table 4.10: Results summary of algae growth in the 80 L PBR and lipid yield

<table>
<thead>
<tr>
<th>Batch</th>
<th>Algae growth period, day</th>
<th>Biomass production, mg algae/L Solution day</th>
<th>Lipid Yield, g lipid/100 g algae</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13</td>
<td>36.9</td>
<td>4.3±0.2</td>
</tr>
<tr>
<td>B</td>
<td>18</td>
<td>36.75</td>
<td>2.27±0.34</td>
</tr>
<tr>
<td>C</td>
<td>14</td>
<td>26.4</td>
<td>4.56±0.63</td>
</tr>
<tr>
<td>D</td>
<td>21</td>
<td>39.13</td>
<td>3.88±0.12</td>
</tr>
<tr>
<td>E</td>
<td>21</td>
<td>10</td>
<td>4.59±0.64</td>
</tr>
<tr>
<td>F</td>
<td>24</td>
<td>7.1</td>
<td>1.38±0.25</td>
</tr>
</tbody>
</table>

Note: Each value shown for the lipid yield is the average of 3 replications ± SD (Standard Deviation).
Figure 4.26: Effect of light and medium sources on algae biomass production and lipid yield in 80 L column PBR.

Figure 4.26 shows that Chlorella vulgaris biomass production in waste water (36.9 mg/L Solution-day) is almost the same as in fresh water (36.75 mg/L Solution-day) using 14,000 LUX Fluorescent light. Chlorella C2 biomass production in waste water (34.29 mg/L Solution-day) is 30% more than that in fresh water (26.4 mg/L Solution-day) using 14,000 LUX Fluorescent light. Chlorella vulgaris biomass production in waste water (7.1 mg/L Solution-day) is 71% of that in fresh water (10 mg/L Solution-day) using 3500 LUX Red-Blue LEDs. The lower productivities are due to the lower Red-Blue light intensities (3500 LUX) vs. 14,000 LUX fluorescent lights. However, Chlorella vulgaris lipid yield in fresh water using LEDs (4.59 g lipid/100 g dry algae) is relatively the same as that using Fluorescent light (4.3 g lipid/100 g dry algae). Also, Chlorella vulgaris lipid yield in waste water using LEDs (1.38 g lipid/100 g dry algae) is 70% of that using Fluorescent light (2.27 g lipid/100 g dry algae):
Table 4.11 compares algae biomass yield (g/l) in waste water produced in the present work with the theoretical yield reported by Christenson et al., 2011. Waste water used in this study was provided by wastewater treatment facility in Dover, New Hampshire. Their characteristics were presented in Chapter 3, Section 3.6.2. The nitrogen concentration was obtained by adding TN of waste water (14mg/l) to TN of the nutrients (72mg/l) to get the TN provided to the algae to grow (86 mg/l). The same procedure was used to obtain the concentration of phosphorous (13.7mg/l). The biomass production of Chlorella vulgaris growth in waste water using Fluorescent light at 8000 LUX was 138.7 mg dry algae/L Solution-day with 15 growth period. That means producing 2080 mg dry algae/L biomass yield (=2.08 g dry algae/l)

Table 4.11: Characterization of typical strong domestic and dairy waste waters with respect to algal nutrients nitrogen and phosphorous (Christenson et al., 2011) vs. characterization of waste water after adding the nutrients of the present work.

<table>
<thead>
<tr>
<th>Types of waste water</th>
<th>Strong domestic</th>
<th>Dairy</th>
<th>Present work waste water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (mg/l)</td>
<td>85</td>
<td>185</td>
<td>86</td>
</tr>
<tr>
<td>Phosphorous (mg/l)</td>
<td>15</td>
<td>30</td>
<td>13.7</td>
</tr>
<tr>
<td>N:P (molar ratio)</td>
<td>13</td>
<td>14</td>
<td>13.88</td>
</tr>
<tr>
<td>Algae biomass production (g/l)</td>
<td>1.4</td>
<td>2.9</td>
<td>2.08</td>
</tr>
</tbody>
</table>

Table 4.12 compares Chlorella vulgaris and Chlorella C2 algae growth and lipid production in 2L PBR versus the scale-up column 80 L PBR in fresh water using fluorescent light. The last two columns list the calculated ratio of the 2L values divided by the 80L values. These ratios are plotted in Figure 4.27

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Table 4.12: Algae growth in small scale versus large scale

<table>
<thead>
<tr>
<th></th>
<th>2L PBR (small scale)</th>
<th>80 L PBR (large scale)</th>
<th>Ratio 2L/80L values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorella vulgaris</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorescent light intensity, LUX</td>
<td>8000</td>
<td>8000</td>
<td>0.571</td>
</tr>
<tr>
<td>Growth period, days</td>
<td>15</td>
<td>14</td>
<td>0.571</td>
</tr>
<tr>
<td>Algae biomass production, mg/L-Solution-day</td>
<td>177.33</td>
<td>130.68</td>
<td>4.8</td>
</tr>
<tr>
<td>Lipid Yield, g lipid/100 g dry algae</td>
<td>4.6</td>
<td>2.79</td>
<td>4.95</td>
</tr>
<tr>
<td>Lipid production, mg lipid/L-Solution-day</td>
<td>8.16</td>
<td>3.65</td>
<td>3.04</td>
</tr>
<tr>
<td>Chlorella C2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorescent light intensity, LUX</td>
<td>8000</td>
<td>8000</td>
<td>0.571</td>
</tr>
<tr>
<td>Growth period, days</td>
<td>14</td>
<td>14</td>
<td>0.571</td>
</tr>
<tr>
<td>Algae biomass production, mg/L-Solution-day</td>
<td>14000</td>
<td>14000</td>
<td>4.8</td>
</tr>
<tr>
<td>Lipid Yield, g lipid/100 g dry algae</td>
<td>4.3</td>
<td>4.56</td>
<td>1.07</td>
</tr>
<tr>
<td>Lipid production, mg lipid/L-Solution-day</td>
<td>1.56</td>
<td>1.2</td>
<td>5.23</td>
</tr>
</tbody>
</table>

Figure 4.27: Comparing small scale to large scale of Chlorella vulgaris and Chlorella C2 growth.
The algae biomass production and the lipid production ratios are greater than 1 indicating that the productivities in the scaled-up column 80 L reactor are lower than the same productivities in the smaller 2L PBR. These findings confirm the fourth hypothesis of the present work.

4.7 FAME ANALYSIS OF BIODIESEL (Objective 6)

4.7.1 FAME PEAKS IDENTIFICATION

The preparation of reference standards was discussed in Chapter 3, Section 3.11.1. The prepared standards at their known concentration were first injected in the GC individually, prior to be mixed in a cocktail, in order to identify their retention time and determine the area under the analyzed peak. Table 4.13 lists each standard, its retention time and the area under each peak.

Table 4.13: Fatty acid methyl ester (FAME) identification.

<table>
<thead>
<tr>
<th>Fatty Acid Methyl Ester (FAME)</th>
<th>Fatty Acid Methyl Ester (FAME) name</th>
<th>Molecular weight, MW</th>
<th>Retention Time, min</th>
<th>Concentration, mg/l</th>
<th>Analyzed area under the peak, arbitrary unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>Methyl palmitate</td>
<td>270.4507</td>
<td>9.7</td>
<td>1.67</td>
<td>1,359,753</td>
</tr>
<tr>
<td>C18:1</td>
<td>Methyl oleate</td>
<td>296.4879</td>
<td>13.18</td>
<td>1.67</td>
<td>399,234</td>
</tr>
<tr>
<td>C18:2</td>
<td>Methyl linoleate</td>
<td>294.4721</td>
<td>13.12</td>
<td>1.67</td>
<td>681,522</td>
</tr>
<tr>
<td>C18:3</td>
<td>Methyl linolenate</td>
<td>292.562</td>
<td>13.3</td>
<td>1.67</td>
<td>719,567</td>
</tr>
<tr>
<td>C18:0</td>
<td>Methyl stearate</td>
<td>298.5038</td>
<td>13.6</td>
<td>1.2</td>
<td>259,342</td>
</tr>
<tr>
<td>C19:0</td>
<td>Methyl Nonadecanoate</td>
<td>312.53</td>
<td>17.8</td>
<td>1.3</td>
<td>402,063</td>
</tr>
</tbody>
</table>
For examples, Figure 4.28 and 4.29 show C18:0 peak and C16:0 respectively when they injected individually and their retention time.

Figure 4.28: Peak of the standard C18:0

Figure 4.29: Peak of the standard C16:0
4.7.2 STANDARD COCKTAIL

Two different cocktails, Cocktail A and Cocktail B, were prepared at different concentration of each standard in order to identify each peak in the cocktail and their retention time. Cocktail A was prepared by mixing 100 µL of each of the following standard: C16:0, C18:0, C18:1, C18:2, C18:3, C19:0. The concentration of each standard in the cocktail was calculated. 3µL of the cocktail mixture was injected in the GC. The results are presented in Table 4.14 and Figure 4.30

Table 4.14: FAME peaks of cocktail A

<table>
<thead>
<tr>
<th>Fatty Acid Methyl Ester (FAME)</th>
<th>Retention Time, min</th>
<th>Concentration of each FAME in the cocktail, mg/l</th>
<th>Analyzed area under the peak, arbitrary unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>9.7</td>
<td>0.278</td>
<td>216139</td>
</tr>
<tr>
<td>C18:1</td>
<td>13.1 and 13.2</td>
<td>0.278</td>
<td>140380 and 202024</td>
</tr>
<tr>
<td>C18:2</td>
<td></td>
<td>0.278</td>
<td></td>
</tr>
<tr>
<td>C18:3</td>
<td></td>
<td>0.278</td>
<td></td>
</tr>
<tr>
<td>C18:0</td>
<td>13.6</td>
<td>0.2</td>
<td>41960</td>
</tr>
<tr>
<td>C19:0</td>
<td>18</td>
<td>0.217</td>
<td>79138</td>
</tr>
</tbody>
</table>

Figure 4.30: FAME peaks of cocktail A
Cocktail B was prepared by mixing 50 µL of each of the following: C16:0, C18:0, C18:1, C19:0 and 100 µL of each of the following: C18:2 and C18:3. The concentration of each standard in the cocktail was calculated. 3µL of the cocktail mixture was injected in the GC. The results are presented in Table 4.15 and Figure 4.28.

<table>
<thead>
<tr>
<th>Fatty Acid Methyl Ester (FAME)</th>
<th>Retention Time, min</th>
<th>Concentration of each FAME in the cocktail, mg/l</th>
<th>Analyzed area under the peak, arbitrary unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>9.8</td>
<td>0.209</td>
<td>163285</td>
</tr>
<tr>
<td>C18:1</td>
<td>13.1 and 13.28</td>
<td>0.209 and 0.418</td>
<td>170082 and 279620</td>
</tr>
<tr>
<td>C18:2</td>
<td>13.28</td>
<td>0.418</td>
<td></td>
</tr>
<tr>
<td>C18:3</td>
<td></td>
<td>0.418</td>
<td></td>
</tr>
<tr>
<td>C18:0</td>
<td>13.7</td>
<td>0.15</td>
<td>62307</td>
</tr>
<tr>
<td>C19:0</td>
<td>18.3</td>
<td>0.0163</td>
<td>74433</td>
</tr>
</tbody>
</table>

Figure 4.31: FAME peaks of cocktail B
Tables 4.13, 4.14 and 4.15 indicate a linear proportionality between the concentration of each standard and the corresponding integrated area under the analyzed peak when injected individually and in each cocktail. Addition cocktail were made to confirm these proportionality.

This linear relation can be considered for C16:0, C18:0 and C19:0. However, C18:1, C18:2, C18:3 appeared as two overlapped peaks when they injected in cocktail A and cocktail B. Chapter 3, Section 3.11.3 showed a cocktail mixture of C18:1, C18:2 and C18:3 in which they appeared also as two peaks only with accumulation area of 667075 at total concentration of 1.67 mg/ml. The cumulative area of C18:1, C18:2 and C18:3 is proportional to their corresponding total concentration when they are in the cocktail.

Retention times may shift slightly if the GC truncated for maintenance or conditioning the GC column. However, the retention time of the peaks will stay in the same order, i.e., the sequence of peaks will always be C16:0, C18:1,2,3, C18:0, C19:0

4.7.3 CONVENTIONAL TRANSESTRIFICATION FAME YIELD

The conventional transestrification is the two-step process; Lipid extraction followed by tranestrification reaction. This tranestrification reaction took place using 0.1 M KOH in methanol at 50 °C (using the hot plate with the magnetic stirrer) for 30 min. This Procedure is shown in Chapter 3, Section 3.10.1. 3 μL of the tranestrifled sample was injected in the GC. Data were transferred from peak 96 to Excel (See Appendix II) for a chromatogram to be created. The biodiesel FAMEs were analyzed as discussed in
Chapter 3, Section 3.11.3. Figure 4.32 shows algae biodiesel FAME peaks produced in the two-step process.

![Figure 4.32: Chlorella vulgaris biodiesel FAME peaks produced in the two-step process (conventional transesterification).](image)

C18:0, C16:0 and C16:1,2,3 were clearly identified from their retention time. A reference standard of C16:1 was not available to identify its peak. However, the peak of C16:1 appears before C16:0 as shown in the literature (Laurens, 2012). The FAME concentration of C16:1 is calculated by proportionality to C16:0. Three unknown peaks appear in Figure 4.29 at time 8.74, 10.72 and 13.47. These unknown peaks are not quantified.

Figure 4.33 shows Chlorella vulgaris biodiesel FAME composition produced in the present work by the traditional two-step process. The figure indicates that the major FAME produced is C18:1,2,3 (42.7%). This is consistent with the results of Mulumba, 2010 and 2012 and Ferrentino, 2007. The total unsaturated FAMEs (C16:1 + C18:1,2,3) is 55.2% and the total saturated FAMEs (C16:0 + C18:0) is 32.6%
Figure 4.33: Composition of Chlorella vulgaris biodiesel FAME produced in the two-step process.

Two replications of the two-step process were done using Chlorella vulgaris batch A and another two replications using Chlorella C2 batch C. Table 4.16 presents the results of biodiesel FAME of these runs. (For batch A and batch C conditions, see Section 4.6). For each FAME, the concentration and the percent are presented.

<table>
<thead>
<tr>
<th>Run</th>
<th>FAME concentration (mg FAME/g of dry algae)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C16:1</td>
</tr>
<tr>
<td>2A1</td>
<td>0.17</td>
</tr>
<tr>
<td>2A2</td>
<td>0.22</td>
</tr>
<tr>
<td>2C1</td>
<td>0.47</td>
</tr>
<tr>
<td>2C2</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Note: number 2 before the letter refer to the two-step process, the letter refer to the batch of algae used for this run (Table 4.9) and the number after the run refer to the replicate.
The total FAME concentrations of Chlorella C2 biodiesel produced in the two-step process in this study (6.62 and 3.08 mg FAMEs per g of dry algae, Table 4.16) are higher than Mulumba, 2010. He reported 2.315 and 1.189 mg of total FAME per g of dry Chlorella C2 biomass for two replications.

The aim of the two-step process in this study was to compare the FAMEs yield versus the one-step process yield.

4.7.4 IN-SITU TRANSESTRIFICATION FAME YIELD

In-situ transestrification is the one-step process to produce microalgae biodiesel through combined algae oil extraction and tranestrification. The process mixes algae and 0.1 M KOH in methanol. A W375 ultrasonicator is used, as discussed in Chapter 3, Section 3.10.2. The effect of the (sonication and reaction) time on the biodiesel FAME production was studied in order to determine the optimum time as shown in Figure 4.34.

Several in-situ runs were done with sonication and reaction time of 1, 5, 8, 10, 12, 20 and 30 minutes. Chlorella vulgaris dry algae obtained from the same batch (batch A, Section 4.6) of the 80 L PBR in fresh water using fluorescent light was used for this experiment.
Figure 4.34 Relative FAME concentration compared to the optimum (Sonication+Reaction) time.

Figure 4.34 shows the FAME concentrations at different (Sonication + Reaction) time relative to the optimum FAME concentration obtained at (Sonication and reaction time of 10 minutes. It indicates that the total FAME concentration increases with increasing the sonication and reaction time until the optimum sonication and reaction time, 10 minutes, is achieved at which maximum total FAME concentration was obtained. When the time increased more than 10 minutes, the total FAME concentration decreased. The reason is that the sonicator releases so much heat which might destruct the FAMEs (C16 – C20) to smaller molecules (C8-C14), which are not detected by the GC. The optimum sonication and reaction time indicated by the present work (10 min) agrees with Ferrentino, 2007. He studied the effect of both the length of ultrasonication time and the effect of the potassium hydroxide concentration on the total mass of FAMEs produced (mg) from Chlorella C2. His study demonstrated that the concentration of 0.1M KOH in methanol produce higher FAME yield compare to 0.2M of that solution and that 10 minutes
ultrasonication was sufficient to provide the highest FAME yield, as shown in the three dimension Figure 4.35, taken from Ferrentino MS thesis. However, Nokongolo, 2012, demonstrated those 20 minutes is the ideal sonication and reaction time through the one-step process.

![Figure 4.35: Total FAMEs, mg with respect to the concentration of the solvent and the (sonication + reaction) time (taken from Ferrentino, 2007)](image)

A sample of 3 μL of algae (Chlorella C2 or Chlorella vulgaris) biodiesel produced in the one step process was injected in the GC and the data was analyzed. Figure 4.36 show algae biodiesel FAME peaks produced in the one-step process.
Figure 4.36: Chlorella vulgaris biodiesel FAME peaks produced in the one-step process (in-situ transestrification), 10 minutes of sonication and reaction time.

Two unknown peaks are shown in Figure 4.36 at times of 7 min. and 11 min., respectively. These two unknown peaks are not quantified. Figure 4.37 shows Chlorella vulgaris biodiesel FAME composition produced in the one-step process (in-situ transestrification). The total unsaturated FAMEs (C16:1 + C18:1,2,3) is 68.3% and the total saturated FAMEs (C16:0 +C18:0) is 29.3%
Figure 4.37: Composition of Chlorella vulgaris biodiesel FAME produced in the one-step process.

Figure 4.38: Comparison of Chlorella vulgaris FAME compositions produced by the 2-step process and by the one-step (in-situ) process.

Figure 4.38 show that chlorella vulgaris biodiesel FAME composition produced in the one-step process is comparable to that in the one-step process. Unknown FAMEs are found in both compositions.

Pure biodiesel (B100) from waste vegetable oil, produced by White Mountain Biodiesel, LLC, was analyzed using the same GC used in this investigation (Hewlett
Packard HP 5890 Series II Gas chromatograph). 1 ml of B100 was diluted with chloroform at a ratio of 1:100 by volume. 3 μL were injected of the sample was injected in the GC. GC data analysis of B100 is listed in Table 4.17. Figure 4.39 shows the FAME composition of B100.

Table 4.17: FAME analysis, composition and concentration of B100

<table>
<thead>
<tr>
<th>FAME</th>
<th>Retention Time, min</th>
<th>Concentration of FAME, mg/ml</th>
<th>Analyzed area under the peak, arbitrary unit</th>
<th>% Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown peak</td>
<td>7.32</td>
<td>Not quantified</td>
<td>15922</td>
<td>0.31</td>
</tr>
<tr>
<td>C16:1</td>
<td>9.6</td>
<td>0.29</td>
<td>23312</td>
<td>0.46</td>
</tr>
<tr>
<td>C16:0</td>
<td>9.86</td>
<td>6.16</td>
<td>501479</td>
<td>9.8</td>
</tr>
<tr>
<td>C18:1, 2, 3</td>
<td>13.34</td>
<td>92.61</td>
<td>3699184</td>
<td>72.3</td>
</tr>
<tr>
<td>C18:0</td>
<td>13.7</td>
<td>39</td>
<td>843052</td>
<td>16.5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>138.06</td>
<td>5115065</td>
<td>99.37</td>
</tr>
</tbody>
</table>

Total FAME concentration was 138.06 mg FAME/ml of B100.

Figure 4.39 B100 FAME Composition
Table 4.18 shows a comparison of Chlorella vulgaris biodiesel FAME composition produced in the two-step process, the one-step process of this study, B100 and literature with respect to the percentage of the saturated and the unsaturated FAMEs.

Table 4.18: Comparison of chlorella vulgaris biodiesel FAME composition (saturated and unsaturated %) of this study and values reported by Gouveia et al., 2009 and Laurens et al., 2012

<table>
<thead>
<tr>
<th>FAME composition</th>
<th>Present study</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Two-step</td>
<td>One-step</td>
</tr>
<tr>
<td>Saturated FAMEs (%)</td>
<td>32.6</td>
<td>29.3</td>
</tr>
<tr>
<td>Unsaturated FAMEs (%)</td>
<td>55.2</td>
<td>68.3</td>
</tr>
<tr>
<td>Unknown %</td>
<td>3.2</td>
<td>1.21</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>98.81</td>
</tr>
</tbody>
</table>

Table 4.19 represents the FAME yield produced in the one-step process of different batches and their replications.

Table 4.19: FAME yield produced in the one-step process from different algae batches

<table>
<thead>
<tr>
<th>Run</th>
<th>FAME concentration (mg FAME/g of dry algae)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C16:1</td>
<td>C16:0</td>
</tr>
<tr>
<td>1A1</td>
<td>4.53</td>
<td>5.24</td>
</tr>
<tr>
<td>1A2</td>
<td>6.1</td>
<td>6.7</td>
</tr>
<tr>
<td>1A3</td>
<td>5.45</td>
<td>5.83</td>
</tr>
<tr>
<td>Average ± SD</td>
<td>55.52±1.13</td>
<td></td>
</tr>
<tr>
<td>1B1</td>
<td>4.52</td>
<td>3.99</td>
</tr>
<tr>
<td>1B2</td>
<td>2.99</td>
<td>1.77</td>
</tr>
<tr>
<td>Average ± SD</td>
<td>44.17±5</td>
<td></td>
</tr>
<tr>
<td>1C1</td>
<td>2.1</td>
<td>2.02</td>
</tr>
<tr>
<td>1C2</td>
<td>2.16</td>
<td>2.02</td>
</tr>
<tr>
<td>1C3</td>
<td>1.65</td>
<td>1.54</td>
</tr>
<tr>
<td>Average ± SD</td>
<td>20.75±2.28</td>
<td></td>
</tr>
<tr>
<td>1D1</td>
<td>2.46</td>
<td>3.67</td>
</tr>
<tr>
<td>1D2</td>
<td>3.01</td>
<td>3.8</td>
</tr>
<tr>
<td>1D3</td>
<td>2.7</td>
<td>3.55</td>
</tr>
<tr>
<td>Average ± SD</td>
<td>39.13±3.2</td>
<td></td>
</tr>
<tr>
<td>1E1</td>
<td>1.92</td>
<td>1.85</td>
</tr>
</tbody>
</table>

161
<table>
<thead>
<tr>
<th></th>
<th>1E2</th>
<th>1.13</th>
<th>1.09</th>
<th>6.83</th>
<th>4.98</th>
<th>14.04</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1E3</td>
<td>1.77</td>
<td>1.72</td>
<td>10.14</td>
<td>6.46</td>
<td>20.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Average ± SD</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>18.13±3.5</strong></td>
</tr>
<tr>
<td>1F1</td>
<td>0.12</td>
<td>0.09</td>
<td>0.62</td>
<td>0.27</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>1F2</td>
<td>0.04</td>
<td>0.03</td>
<td>0.14</td>
<td>0</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>1F3</td>
<td>0.07</td>
<td>0.06</td>
<td>0.31</td>
<td>0</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Average ± SD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>0.59±0.47</strong></td>
</tr>
</tbody>
</table>

Note that number 1 before the letter refer to the one-step process (in-situ process) and the letter refer to the batch of algae used for this run (Table 4.9) and the number after the run refer to the replicate.

Figure 4.40: The effect of light source and medium source on algae average total FAME yield in large scale.

Figure 4.40 shows the average of total biodiesel FAME yield produced in the one-step process. It indicates that the total FAME yield of waste water (44.17 mg FAME/g dry algae) is 80% of that of fresh water (55.52 mg FAME/g dry algae) for Chlorella vulgaris. Total FAME yield of waste water (39.13 mg FAME/g dry algae) is 88% more than that of fresh water (20.75 mg FAME/g dry algae) for Chlorella C2. These indicate that waste
water is suitable to use for growing algae in order to reduce fresh water usage and achieve better economic for biodiesel production. Moreover, total FAME yield of fresh water and Red-Blue LEDs (18.13 mg FAME/g dry algae) is 33% of that of fresh water, Fluorescent (55.52 mg/g of dry algae) for Chlorella vulgaris, which indicates Red-Blue LEDs is comparable to Fluorescent in large scale PBRs since the light intensity of the Red-Blue LEDs jacket is one fourth of the Fluorescent light intensity. However, waste water with the low light intensity of the Red-Blue LEDs for Chlorella vulgaris produce low FAME yield (0.59 mg FAME/g of dry algae).

4.8 BIODIESEL PHYSICAL PROPERTIES ESTIMATION (Objective 6)

Biodiesel fuel or FAMEs are to be used for energy security, i.e., lower the dependency on imported crude oil, and to reduce the greenhouse gas emissions of the transportation and aviation sectors. This requires knowledge of the physical properties of biodiesel, e.g., density, heat of combustion, Cetane number, and so on. These properties are not the same as the petroleum diesel properties. One of the most important properties is density (or specific gravity). When blending biodiesel with jet fuel to produce bio-jet fuel it is important to realize that biodiesel is denser than diesel. There is often an upper limit of the density of the jet fuel. Knowledge of the biodiesel density is crucial to making sure the blend does not exceed the specifications of jet fuel. Cetane number is an important biodiesel property, but its measurement is not a simple process and is time consuming. Accurate knowledge of biodiesel density would permit the use of Cetane estimation methods available in the Literature.
Ramirez-Verduzco et al, 2012 stated four new empirical correlations to calculate the Cetane number, kinematic viscosity, density, higher heating value of fatty acid methyl esters. Their correlations related the properties of the FAMEs to their molecular weight and the degree of unsaturation.

The Cetane number of each FAME is obtained from equation 4.3

\[ \Phi_i = -7.8 + 0.302 \times M_i - 20 \times N \]  

Equation 4.3

Where \( \Phi_i \) is the Cetane number of the \( i \)th FAME, \( M_i \) is the molecular weight of the \( i \)th FAME and \( N \) is the the number of double bonds (DB) in a given FAME.

The kinematic viscosity as a function of \( M_i \) and \( N \) is expressed in equation 4.4

\[ \ln(v_i) = -12.503 + 2.496 \times \ln(M_i) - 0.178 \times N \]  

Equation 4.4

Where \( v_i \) is the kinematic viscosity at 40 °C of the \( i \)th FAME in mm²/s.

The density of saturated and unsaturated FAMEs is expressed in equation 4.5

\[ \rho_i = 0.8463 + \frac{4.9}{M_i} + 0.0118 \times N \]  

Equation 4.5

Where \( \rho_i \) is the density at 20 °C of the \( i \)th FAME in g/ml.

The higher heating value of FAMEs can be calculated from equation 4.6

\[ \delta_i = 46.19 - \frac{1794}{M_i} - 0.21 \times N \]  

Equation 4.6

Where \( \delta_i \) is the higher heating value of the \( i \)th FAME in KJ/g.

Ramirez-Verduzco et al, 2012 demonstrated that the physical properties of biodiesel can be estimated from the individual physical properties of FAMEs using appropriate mixing rules. Equation 4.7 Represent the general expression used for their studies.

\[ f_b = \sum_{i=1}^{n} z_i \times f_i \]  

Equation 4.7
Where \( f \) is a function that represents any physical property (the subscript \( b \) and \( i \) refer to the biodiesel and the pure \( i \)th FAME, respectively), \( z_i \) is the mass fraction of the \( i \)th FAME.

The present study used these empirical equations and the mixing rule in order to predict the physical properties of B100 and compare with measured values. Tables 4.20 and 4.21 summarize the results.

**Table 4.20: Predicted physical properties of individual biodiesel (B100) FAMES.**

<table>
<thead>
<tr>
<th>FAME</th>
<th>Molecular weight ((M_i))</th>
<th>Degree of saturation ((N))</th>
<th>Density at 20 °C ((\rho_i)), g/ml</th>
<th>Kinematic viscosity at 40 °C (\ln(\nu_i)), mm²/s</th>
<th>Cetane number ((\Phi_i))</th>
<th>Higher heating value ((\delta_i)), KJ/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:1</td>
<td>268.4348</td>
<td>1</td>
<td>0.8764</td>
<td>1.2781</td>
<td>53.2673</td>
<td>39.2968</td>
</tr>
<tr>
<td>C16:0</td>
<td>270.4507</td>
<td>0</td>
<td>0.8644</td>
<td>1.4748</td>
<td>73.8761</td>
<td>39.5566</td>
</tr>
<tr>
<td>C18:1</td>
<td>296.4879</td>
<td>1</td>
<td>0.8746</td>
<td>1.5262</td>
<td>61.7393</td>
<td>39.9292</td>
</tr>
<tr>
<td>C18:2</td>
<td>294.4721</td>
<td>2</td>
<td>0.8865</td>
<td>1.3312</td>
<td>41.1306</td>
<td>39.6777</td>
</tr>
<tr>
<td>C18:3</td>
<td>292.4562</td>
<td>3</td>
<td>0.8985</td>
<td>1.1361</td>
<td>20.5218</td>
<td>39.4257</td>
</tr>
<tr>
<td>C18:0</td>
<td>298.5038</td>
<td>0</td>
<td>0.8627</td>
<td>1.7212</td>
<td>82.3481</td>
<td>40.1800</td>
</tr>
</tbody>
</table>

**Table 4.21: Predicted and measured physical properties of total B100 FAMES**

<table>
<thead>
<tr>
<th>FAME conc. mg FAME/g algae</th>
<th>Mass fraction (z_i)</th>
<th>(z_i \times \rho_i) g/ml</th>
<th>(z_i \times M_i) g/gmol</th>
<th>(z_i \times \ln(\nu_i))</th>
<th>(z_i \times \Phi_i) Cetane No. dimensionless</th>
<th>(z_i \times \delta_i) KJ/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:1</td>
<td>0.29</td>
<td>0.0021</td>
<td>0.0018</td>
<td>0.5638</td>
<td>0.0027</td>
<td>0.1119</td>
</tr>
<tr>
<td>C16:0</td>
<td>6.16</td>
<td>0.0446</td>
<td>0.0386</td>
<td>12.0662</td>
<td>0.0658</td>
<td>3.2960</td>
</tr>
<tr>
<td>C18:1,2,3</td>
<td>92.61</td>
<td>0.6707</td>
<td>0.5946</td>
<td>197.5162</td>
<td>0.8929</td>
<td>27.5882</td>
</tr>
<tr>
<td>C18:0</td>
<td>39.01</td>
<td>0.2825</td>
<td>0.2437</td>
<td>84.3386</td>
<td>0.4863</td>
<td>23.2665</td>
</tr>
<tr>
<td>Predicted biodiesel property</td>
<td>138.07</td>
<td>0.8788</td>
<td>294.4848</td>
<td>1.4477 (* v = 4.25 ) cSt</td>
<td>54.2625</td>
<td>39.8135</td>
</tr>
<tr>
<td>Experimental values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Property name</td>
<td>Total FAME conc.</td>
<td>B100 density</td>
<td>B100 Mol wt</td>
<td>Cetane No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The present work measured density and heating value of B100 are 0.853 g/ml (Table 4.25) and 40.824 KJ/g (Figure 4.44), respectively. These were included in Table 4.21. The experimental and predicted values are in good agreement. This provides confidence in the Ramirez-Verduzco prediction equations. Therefore, the present work adopted Ramirez-Verduzco formulas and the mixing rule (Equation 4.3, 4.4, 4.5, 4.6 and 4.7) to predict the physical properties of each FAME and the total in-situ algae biodiesel produced in the present work. The data of the FAME concentrations produced in the one-step process (in-situ process) (Table 4.19) are used to predict the physical properties of Chlorella vulgaris and Chlorella C2 biodiesel. The results are shown in Table 4.22

<table>
<thead>
<tr>
<th>Batch</th>
<th>$\rho_b$, g/ml at 20 °C</th>
<th>$M_b$, g/gmol at 40 °C</th>
<th>$\nu_b$, mm$^2$/s at 40 °C</th>
<th>$\phi_h$ Cetane No, dimensionless</th>
<th>$\delta_b$, KJ/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A1</td>
<td>0.878</td>
<td>290.89</td>
<td>4.158</td>
<td>54.202</td>
<td>39.74</td>
</tr>
<tr>
<td>1B1</td>
<td>0.877</td>
<td>290.73</td>
<td>4.261</td>
<td>57.083</td>
<td>39.77</td>
</tr>
<tr>
<td>1C3</td>
<td>0.876</td>
<td>291.68</td>
<td>4.323</td>
<td>58.038</td>
<td>39.8</td>
</tr>
<tr>
<td>1D3</td>
<td>0.879</td>
<td>291.44</td>
<td>4.122</td>
<td>52.841</td>
<td>39.74</td>
</tr>
<tr>
<td>1E1</td>
<td>0.876</td>
<td>291.41</td>
<td>4.324</td>
<td>58.257</td>
<td>39.8</td>
</tr>
<tr>
<td>1F1</td>
<td>0.878</td>
<td>290.66</td>
<td>4.191</td>
<td>55.251</td>
<td>39.75</td>
</tr>
</tbody>
</table>

The predicted physical properties of Chlorella vulgaris and Chlorella C2 biodiesel satisfy the European standard EN14214. EN 14214 standards are either the same or tighter than the US ASTM 6752 standards (See Table 2.21). Therefore, a biodiesel sample that satisfies the density, viscosity, kinematic viscosity, Cetane number and higher heating values of the European Standards EN 14214 will also satisfy ASTM 6752.
4.9 EFFECT OF NITROGEN DEPRIVATION ON FAME PRODUCTION

(Objective 8)

The present work studied one of lipid triggering method, which is nitrogen deprivation. Nitrogen is one of the most important nutrients for algae growth. As the nitrogen concentration decreases in the growth medium, the algae biomass production accordingly decreases. But the FAME yield (mg FAME/g of dry algae) increases. However, the challenge is to study the effect of nitrogen starvation on the biodiesel FAME production (mg FAME/L Solution-day), which is the product of the algae biomass production by the FAME yield.

For this experiment, Chlorella vulgaris algae were grown in four of the 2L PBR which were operated at the same time using fluorescent light at 8000 LUX. The same nutrients were added to each PBR (see Table 3.4 for the required nutrients of algae growth) with different amounts of KNO$_3$ in each solution; 1.05, 0.8, 0.525and 0.263g. Thus KNO$_3$ concentrations in the four runs were 0.525, 0.4, 0.2625 and 0.1315 g/l. The medium with 0.525 g/l of KNO$_3$ is the control or the standard medium. The growth of each run was monitored by the turbidity measurements, cell counts, PH, nitrate and nitrite (Data are not included). The most important indicator was observed during this experiment was the nitrate measurements. The nitrate measured reached zero at the end of the exponential phase. Then, the cells stopped doubling and start to store energy, which resulted in increasing the lipids. Each culture was harvested about two days after the nitrate reached zero.
Biodiesel FAMEs were produced through the one-step process (in-situ transestrification). The data were analyzed using the GC. The results are shown in Table 4.23.

Table 4.23: Chlorella vulgaris nitrogen deprivation results

<table>
<thead>
<tr>
<th>KNO₃, g/L</th>
<th>KNO₃, mM</th>
<th>Algae biomass production, g/L Solution-day</th>
<th>FAME yield, mg FAME/g dry algae</th>
<th>FAME production, mg FAME/L Solution-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.525</td>
<td>5.19</td>
<td>0.131</td>
<td>8.59</td>
<td>1.13</td>
</tr>
<tr>
<td>0.4</td>
<td>3.96</td>
<td>0.098</td>
<td>24.42</td>
<td>2.4</td>
</tr>
<tr>
<td>0.2625</td>
<td>2.6</td>
<td>0.082</td>
<td>34.7</td>
<td>2.85</td>
</tr>
<tr>
<td>0.1315</td>
<td>1.3</td>
<td>0.044</td>
<td>16.1</td>
<td>0.7</td>
</tr>
</tbody>
</table>

FAME production, mg/L Solution-day, in Tables 4.19 and 4.20 was calculated by multiplying the algae biomass production, mg dry algae/L Solution-day, and the FAME yield, mg FAME/g dry algae. FAME production should be a better indicator than the FAME yield as indicated in section 4.1.5 about the lipid production.

Figure 4.41: Effect of potassium nitrate concentration on Chlorella vulgaris FAME yield, graph (a) and FAME production, graph (b).
Figure 4.41 shows that 50% decrease of potassium nitrate concentration in the medium (from 5.19 to 2.6 mM) resulted in fourfold increase in the FAME yield (from 8.59 to 34.7 mg FAME/g of dry algae) and two and a half fold increase in the FAME production (from 1.13 to 2.85 mg FAME/L Solution-day). However, 75% decrease of potassium nitrate concentration (from 5.19 to 1.3 mM) resulted in only double the FAME yield (from 8.59 to 16.1 mg FAME/g of dry algae) and decreasing the FAME production by 40% (from 1.13 to 0.7 mg FAME/L Solution-day). This may be caused by the reduction in the biomass production at 1.3 mM of KNO₃. Thus, it is important to calculate the FAME production to investigate the optimum biodiesel FAME production.

The same experiment was repeated to study the effect of nitrogen deprivation on Chlorella C2. The concentration of potassium nitrate (g/l) in Chlorella C2 media was reduced to half and quarter of the control or the standard media (0.525 g/l). KNO₃ concentrations in the three runs were 0.525, 0.2625 and 0.1315 g/l. The results are shown in Table 4.24

<table>
<thead>
<tr>
<th>KNO₃, g/l</th>
<th>KNO₃, mM</th>
<th>Algae biomass production, g/L Solution-day</th>
<th>FAME yield, mg FAME/g dry algae</th>
<th>FAME production, mg FAME/L Solution-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.525</td>
<td>5.19</td>
<td>0.29</td>
<td>5.05</td>
<td>1.46</td>
</tr>
<tr>
<td>0.2625</td>
<td>2.6</td>
<td>0.24</td>
<td>8.07</td>
<td>1.49</td>
</tr>
<tr>
<td>0.1315</td>
<td>1.3</td>
<td>0.16</td>
<td>22.84</td>
<td>3.65</td>
</tr>
</tbody>
</table>
Figure 4.42: Effect of potassium nitrate concentration on Chlorella C2 FAME yield, graph (a) and FAME production, graph (b).

Figure 4.42 indicates that the FAME yield and FAME production increase with the decrease of the KNO₃ concentration. The results show that 75% decrease of potassium nitrate concentration in the Chlorella C2 medium with respect to the control resulted in more than fourfold increase in the FAME yield (from 5.05 to 22.84 mg FAME/g of algae) and twofold and half increase in the FAME production (from 1.46 to 3.65 mg FAME/L Solution-day). Therefore, total biodiesel FAME production can be increased by the reduction of nitrogen concentration in the medium. The conclusions from these results are significantly in agreement with that of Converti et al., 2009 (see Chapter 2, Section 2.7) considering that their results are plotted as lipid content and lipid production.

Note that The FAME yields (mg FAMEs/g dry algae) produced in this work experiments are the total concentration of the biodiesel FAMEs (C16:0, C16:1, C18:0,
The detailed results of the biodiesel FAMEs concentrations are summarized in Appendix IV.

4.10 BIODIESEL-JET FUEL BLEND PROPERTIES (Objectives 9 and 10)

Biodiesel (B100) from waste vegetable oil was blended with aviation jet fuel to produce bio-jet fuel. Blends of different volume ratios of biodiesel to jet fuel were prepared. The following properties of the blend were determined; specific gravity, freezing point and heat of combustion. Correlations between each property and the blending ratio were developed.

4.10.1 SPECIFIC GRAVITY

Specific gravity of the pure fuels; biodiesel from waste vegetable oil (B100) and aviation petroleum-based jet fuel were determined by evaluating the density of each fuel with respect to the density of water (1 g/ml) using equation 3.4. Certain volume of each fuel was massed to determine its density. Table 4.25 lists these results.

<table>
<thead>
<tr>
<th>Fuel</th>
<th>Density, g/ml</th>
<th>Specific gravity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>B100</td>
<td>0.853</td>
<td>0.853</td>
<td>Comparable to the biodiesel range (0.86-0.9) of ASTMD</td>
</tr>
<tr>
<td>Jet fuel</td>
<td>0.791</td>
<td>0.791</td>
<td>Comparable to JP-A range (0.775-0.840), Exxon, 2005</td>
</tr>
</tbody>
</table>
Chlorella vulgaris biodiesel produced in this study by in-situ transestrification was not easy to be handled (it was difficult to measure its volume). Chlorella vulgaris biodiesel was blended with 10 ml of jet fuel. The mass of jet fuel and the mass of the blend were measured. Hence, the mass of vulgaris biodiesel was calculated from mass balance, as follows:

The mass of vulgaris biodiesel = the mass of the blend – the mass of jet fuel

\[
= 19.3627 \text{g} - 7.91 \text{g} = 11.4527 \text{g}
\]

The mass fractions of jet fuel and vulgaris biodiesel were calculated to be 0.409 and 0.591 respectively.

The volume of blend (19.3627 g) was measured to be 19.2 ml. Thus, the density of the blend can be calculated and the specific gravity of the blend was calculated to be 1.008.

Equation 3.5 was used to determine the specific gravity of vulgaris biodiesel as follows:

\[
\frac{1}{SG_{\text{blend}}} = \frac{m_{\text{jet fuel}}}{SG_{\text{jet fuel}}} + \frac{m_{\text{vulgaris biodiesel}}}{SG_{\text{vulgaris biodiesel}}}
\]

\[
SG_{\text{vulgaris biodiesel}} = 1.24
\]

The measured and calculated values are summarized in Table 4.26

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume, ml</th>
<th>Mass g</th>
<th>Density, g/ml</th>
<th>Mass fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vulgaris BD</td>
<td>11.4527</td>
<td>1.24</td>
<td>0.591</td>
<td></td>
</tr>
<tr>
<td>Jet Fuel</td>
<td>10</td>
<td>7.91</td>
<td>0.791</td>
<td>0.409</td>
</tr>
<tr>
<td>Blend</td>
<td>19.2</td>
<td>19.3627</td>
<td>1.008</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Specific gravity of Chlorella vulgaris biodiesel produced in this study was unrealistically larger than the specific gravity range of biodiesel determined by ASTMD-287 (0.86-0.9). This challenge was as an obstacle in determine the properties of this blend. Mallick et al.,
2012 demonstrated that vulgaris biodiesel had density $881 \text{kg/m}^3$ (See Table 2.16), which implies a specific gravity of 0.881.

Generally, the density (and specific gravity) of the biodiesel increases with molecular weight increase, Akbar et al., 2009. The calculated high density of Chlorella vulgaris biodiesel may indicate the presence of high molecular weight components, which are not detected by the GC FAME analysis.

Biodiesel from waste vegetable oil (B100) was blended with Jet fuel with a ratio of 50:50 by volume. The measured and calculated values are summarized in Table 4.27.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume, ml</th>
<th>Mass g</th>
<th>Density, g/ml</th>
<th>Mass fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>B100</td>
<td>10</td>
<td>8.53</td>
<td>0.853</td>
<td>0.5269</td>
</tr>
<tr>
<td>Jet Fuel</td>
<td>10</td>
<td>7.91</td>
<td>0.791</td>
<td>0.4886</td>
</tr>
<tr>
<td>Blend</td>
<td></td>
<td>16.19</td>
<td>0.81 (Eq. 3.5)</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume, ml</th>
<th>Mass g</th>
<th>Density, g/ml</th>
<th>Mass fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>B100</td>
<td>10</td>
<td>8.53</td>
<td>0.853</td>
<td>0.5269</td>
</tr>
<tr>
<td>Jet Fuel</td>
<td>10</td>
<td>7.91</td>
<td>0.791</td>
<td>0.4886</td>
</tr>
<tr>
<td>Blend</td>
<td></td>
<td>16.19</td>
<td>0.81 (Eq. 3.5)</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

4.10.2 FREEZING POINT

The measured freezing point of B100 was $0^\circ\text{C}$ while the freezing point of pure aviation jet fuel was measured to be $-52^\circ\text{C}$.

Different blends of B100 and jet fuel were prepared at volumetric ratios of B100: jet fuel of 40:60, 30:70, 20:80, 10:90, i.e., the volume fraction (x) of B100 in the blend were 0.4, 0.3, 0.2 and 0.1 respectively. The freezing points of these blends were determined using the method in Chapter 3, Section 3.13.2. Figure 4.39 shows the measured blend freezing point, $^\circ\text{C}$ versus the volume fraction of biodiesel (B100). The data of the freezing point
(y) versus the volume fraction of B100 (x) was fitted using a second order equation. The resulting correlation is

\[ FP = y = -85.207x^2 + 132.06x - 47.225, \quad R^2 = 0.9504 \]

The fitted equation is plotted as a solid line on the graph in Figure 4.43.

Figure 4.43: Freezing point of bio-jet fuel with different ratios

4.10.3 HEAT OF COMBUSTION

The heat of combustion of the pure fuels and the blends were determined using the bomb calorimeter (see Chapter 3, Section 3.13.3). Schlagermann et al, 2012 demonstrated that lipid production leads to an increase of the heating value of the biomass from 20 MJ/kg (20 KJ/g= 4777 cal/g = 8598 Btu/lbm) for oil-poor algae (between 20% to 30%) to 30 MJ/kg (30 KJ/g= 7165 cal/g = 12,898 Btu/lbm) for oil-rich algae (50 % dry algae weight) and the heating value of algal biodiesel is 41 MJ/kg. (41 KJ/g dry algae= 9793 cal/g = 17,627 Btu/lbm) The measured heating value of Chlorella vulgaris algae biomass produced in this study was 15.95 KJ/g (= 3810 cal/g = 6857
Btu/lbm) while the heating value of vulgaris biodiesel produced in the one-step process is determined to be 19.89 KJ/g (= 4751 cal/g = 8581 Btu/lbm).

The heat of combustion of pure B100 and jet fuel were measured to be 40.8 KJ/g (= 4751 cal/g = 17,541 Btu/lbm) and 47.08 KJ/g (= 11,241 cal/g = 20,245 Btu/lbm), respectively. Blends of B100 and jet fuel were prepared at ratios of B100 to jet fuel of 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 80:20, i.e., the volume fraction (x) of B100 in the blend were 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.8, respectively. The heat of combustion of these blends was measured. The relation between the heating values and the volume fraction of biodiesel was investigated as shown in Figure 4.44.

The bio-jet blend data of the heating value \( y \) (KJ/g) versus the volume fraction of B100 \( x \) in the blend was fitted using a linear equation. The resulting correlation is

\[
HV = y = -6.1387x + 46.397, \quad R^2 = 0.7771
\]

![Figure 4.44: Heating values of bio-jet fuel with different ratio](image)

The bio-jet blend data of the heating value \( y \) (KJ/g) versus the volume fraction of B100 \( x \) in the blend was fitted using a linear equation. The resulting correlation is

\[
HV = y = -6.1387x + 46.397, \quad R^2 = 0.7771
\]
This Equation is very useful in estimating the heating value of bio-jet fuel produced by blending B100 and petroleum-based aviation jet fuel.

4.11 DESIGN OF EXPERIMENTS AND STATISTICAL ANALYSIS

4.11.1 ONE WAY ANALYSIS OF VARIANCE

Figure 4.45: Actual by predicted lipid production (mg lipid/L Solution-day) plot (from JMP output)

Figure 4.45 shows the actual lipid production (mg lipid/L Solution-day) vs. the predicted lipid production with P value equal 0.0059. P value less than 0.05 indicates that the response (lipid production) is significantly affected by the treatments; algae species, light source and water source.
Table 4.28: Effect test of ANOVA analysis (from JMP output)

<table>
<thead>
<tr>
<th>Source</th>
<th>Nparm</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae species</td>
<td>1</td>
<td>1</td>
<td>15.636148</td>
<td>10.6654</td>
<td>0.0029*</td>
</tr>
<tr>
<td>Light Source</td>
<td>2</td>
<td>2</td>
<td>10.516592</td>
<td>3.5876</td>
<td>0.0410*</td>
</tr>
<tr>
<td>Algae species*Light Source</td>
<td>2</td>
<td>2</td>
<td>6.140748</td>
<td>2.0943</td>
<td>0.1420</td>
</tr>
<tr>
<td>Water Source</td>
<td>1</td>
<td>1</td>
<td>2.181585</td>
<td>1.4881</td>
<td>0.2327</td>
</tr>
<tr>
<td>Algae species*Water Source</td>
<td>1</td>
<td>1</td>
<td>3.522784</td>
<td>2.4029</td>
<td>0.1323</td>
</tr>
</tbody>
</table>

Table 4.28 shows that the P values of algae species and light source are 0.0029 and 0.041. Both are less than 0.05. Therefore, they have significant effects on lipid production.

Water source has P value (0.2327) higher than 0.05. Hence, water source has no significant effect on lipid production, which means that waste water results are promising and comparable to fresh water results. Water source is involved in the interaction profile as shown in figure 4.46.

![Figure 4.46: Prediction profiler and the desirability profile (from JMP output)](image-url)

Figure 4.46: Prediction profiler and the desirability profile (from JMP output)
Figure 4.46 show the prediction profile and the desirability function. It indicated that the most desirable treatments are Chlorella vulgaris algae species, waste water as water source and Red-Blue LEDs as light source.

Figure 4.47: Interaction profile (from JMP output)
Note that mix refers to Red-Blue LEDs

Figure 4.47 show the interaction profile which indicates that the highest lipid production can be obtained by Chlorella vulgaris growth in waste water using mix (mix refers to Red-Blue LEDs). However the highest Chlorella C2 lipid production can be obtained in fresh water using red LEDs.
4.11.2 EACH PAIR STUDENT’S T TEST

Analysis the results of the Each Pair Student’s t Test showed the following:

![Graph showing lipid production by algae species](image)

Figure 4.48: One way analysis of lipid production (mg lipid/L Solution-day) by algae species (from JMP output)

Figure 4.48 shows that by clicking on the top circle, the circle of Chlorella vulgaris turned to red and the circle of Chlorella C2 turned into grey and the circles were separated. This means that algae species has significant effect on lipid production. Chlorella vulgaris produced higher lipid production than Chlorella C2.
Figure 4.49: One way analysis of lipid production (mg lipid/L Solution-day) by light source (from JMP output)

Figure 4.49 shows that by clicking the top circle, the three circles appeared in red color and they are interacted. The circle of Red and mix (Red-Blue) LEDs seem to be overlapped and produce higher lipid production than Fluorescent.

Figure 4.50: One way analysis of lipid production (mg lipid/L Solution-day) by water source (from JMP output)
Figure 4.50 shows that by clicking on the top circle, the two circles turned into red color and they are interacted. This indicated that the medium source does not significantly affect the lipid production. Therefore, the lipid production of waste water are comparable to that of fresh water.
CHAPTER V

5 CONCLUSIONS

All the Objectives of the present study to produce bio-jet fuel from microalgae have been completed. The conclusions from the experimental results and theoretical modeling developed in the present work are:

1. Minimizing the energy requirements for microalgae growth (and bio-jet fuel production) was accomplished by replacing fluorescent lights with light emitting diodes (LEDs). Illuminating the PBR with LEDs resulted in 48.5% energy saved and showed increase in the lipid production. Chlorella vulgaris showed the highest lipid production, 12.44mg lipid/L Solution-day in fresh water, using Red-Blue LEDs while Chlorella C2 showed the highest lipid productions, 6.27 and 6.9 mg/L Solution-day in fresh water and waste water respectively, using Red LEDs. In addition to, Using LEDs makes the process greener since 23.7 and 22.3 lbs of CO$_2$ could be avoided when growing algae in a 2L PBR for 14 days using Red and Red-Blue LEDs respectively. These are equivalent to CO2 avoidances of 0.85 and 0.8 lbs CO$_2$/L-day, or 0.38 and 0.35 kg CO$_2$/L-day.

2. Reducing fresh water footprint in algae growth for bio-jet fuel production was accomplished by replacing fresh water with waste water. The algae production and lipids productivity in municipal waste water was comparable to algae growth in fresh water using the same nutrients. In large scale PBR (80 L column PBR), waste water produced almost the same biomass production (36.9 mg/L Solution-day) as in fresh water (36.75 mg/L Solution-day) for Chlorella vulgaris using
Fluorescent light. Chlorella C2 biomass production in waste water (34.29 mg/L Solution-day) is 30% more than that in fresh water (26.4 mg/L Solution-day) using Fluorescent light.

3. The highest light capture efficiency (photosynthetic efficiency) of 12.9 % was obtained when growing Chlorella C2 in Fresh water using Red LEDs. A photosynthetic efficiency of 8.42% was be obtained when growing Chlorella vulgaris in Fresh water using Red-Blue LEDs.

Microalgae are efficient in capturing CO\textsubscript{2}. The highest carbon capture efficiency of 13.15% was obtained by growing Chlorella C2 in Fresh water using Red LEDs.

4. Light intensity is an important parameter in microalgae growth. Algae biomass and lipid production increase as the intensity increases of all light sources.

5. A kinetics model of a semi-batch PBR was developed to determine the microalgae specific growth rate. This kinetics model was similar to Huesemann, 2009 and Borja, 2008. The maximum specific growth rate, $\mu_{\text{max}}$ was evaluated for each Chlorella algae studied (vulgaris and C2) in each growth medium (fresh and waste water) and each light source (fluorescent, red LEDs and red-blue LEDs). It is concluded either that the exponential phase of all runs should start at the same initial turbidity or that the algae growth kinetics during the lag phase is of importance. The value of specific growth rate alone is not enough to reach a conclusion about the algae growth.

6. The biomass and lipid productivities in large scale PBR (80 L column reactor) are lower than the same productivities in the smaller two Liters PBR due to self-shading.
7. One-step process (in-situ transesterification process) to produce biodiesel resulted in higher FAME yield (55.52 and 39.13 mg FAME/one g dry algae for Chlorella vulgaris and Chlorella C2 respectively) than two-step process (4.03 and 6.62 mg FAME /one g dry algae for Chlorella vulgaris and Chlorella C2 respectively). In addition, the two-step process is time consuming, and uses hazardous and expensive solvent as Hexane. All these disadvantages were avoided in the one-step process.

8. Biodiesel properties prediction was accomplished using the biodiesel FAMEs concentrations obtained from the GC analysis. The predicted density (0.8788) and heat of combustion of B100 (39.81 KJ/g) were comparable to measured density (0.853) and heat of combustion (40.82 KJ/g) in the present work. The predicted density, kinematic viscosity, Cetane number and higher heating value satisfied the European standard EN14214.

9. This study was successful in increasing the FAME production using one of the lipid triggering method, Nitrogen deprivation. 50% decrease of potassium nitrate concentration in the Chlorella vulgaris medium (from 5.19 to 2.6 mM) resulted in two and half fold increase in the Chlorella vulgaris FAME production (from 1.13 to 2.85 mg FAME/L Solution-day). 75% decrease of potassium nitrate concentration in the Chlorella C2 medium with respect to the control resulted in twofold and half increase in the FAME production (from 1.46 to 3.65 mg FAME/L Solution-day). Therefore, total biodiesel FAME production can be increased by moderate reduction of nitrogen concentration in the medium.
10. This study investigated different correlations between the physical properties (heating values and freezing point) and the volume fraction of biodiesel in biodiesel and jet fuel blend.

11. JMP Statistical analysis using one way ANOVA showed p-value of 0.0059 and the test effect concluded that algae species and light source have significant effect on the lipid production. The interaction profile showed that the best conditions are Chlorella vulgaris algae species, Red-Blue LEDs as light source, and waste water as water source.

The Objectives and Conclusions of the present work are summarized in Table 5.1

Table 5.1: Project Objectives and Conclusions

<table>
<thead>
<tr>
<th>No.</th>
<th>Objective Description</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Investigate minimizing the energy requirements for microalgae growth by replacing fluorescent lights with light emitting diodes (LEDs).</td>
<td>Using LEDs resulted in 48.5% saving energy. Highest Chlorella vulgaris lipid production using Red-Blue LEDs. Highest Chlorella C2 lipid production using Red LEDs.</td>
</tr>
<tr>
<td>2</td>
<td>Investigate the use of municipal waste water in growing microalgae to reduce fresh water use.</td>
<td>Waste water is promising for microalgae growth and comparable to fresh water especially for large scale.</td>
</tr>
<tr>
<td>3</td>
<td>Study the effect of light intensity on algae oil production.</td>
<td>Light intensity increases the lipid production for all light sources.</td>
</tr>
<tr>
<td>4</td>
<td>Kinetic study of microalgae growth</td>
<td>Kinetics study of the lag phase is of importance. Value of specific growth rate alone is not enough to reach a conclusion about the algae growth.</td>
</tr>
<tr>
<td>5</td>
<td>Investigate the scale up of microalgae growth in fresh water and waste water from 2 Liters to 80 Liters using fluorescent light and red-blue LEDs.</td>
<td>Scale up microalgae growth resulted in low biomass and lipid production.</td>
</tr>
<tr>
<td>6</td>
<td>Determine the Fatty Acid Methyl Ester (FAME) composition of the microalgae biodiesel and use it to predict its physical properties.</td>
<td>FAME composition was successfully used to predict the physical properties, which satisfied the European and the American standard.</td>
</tr>
<tr>
<td></td>
<td>Study the one-step production of biodiesel using in situ algal biomass transestrification process to reduce production time and cost.</td>
<td>One-step process resulted in Higher FAME yield (up to 56.82 mg FAME/1 g algae), lower cost, less time than two-step process</td>
</tr>
<tr>
<td>----</td>
<td>----------------------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>8</td>
<td>Study the nitrogen starvation effect on the fatty acid methyl esters (FAMEs) concentration.</td>
<td>Nitrogen starvation resulted in increasing FAME production in both species; Chlorella vulgaris and Chlorella C2</td>
</tr>
<tr>
<td>9</td>
<td>Investigate biodiesel blending with jet-fuel in order to obtain bio-jet fuel.</td>
<td>The blending was successfully accomplished</td>
</tr>
<tr>
<td>10</td>
<td>Determine the properties of the blend of different biodiesel: jet-fuel ratios. The properties include specific gravity, freezing points, and heat of combustion.</td>
<td>Useful correlations between the physical properties (heating values and freezing point) and the volume fraction of biodiesel in biodiesel and jet fuel blend were investigated</td>
</tr>
</tbody>
</table>
CHAPTER VI

6 RECOMMENDATIONS FOR FUTURE WORK

The following investigations should to be further studied in detail in order to address questions or challenges encountered in the present work. These questions/challenges are important to further understand the best and most economical conditions to produce bio-jet fuel from microalgae to the solvent phase. However, most of these questions and/or difficulties were not among the main objectives of the present work.

1. The same nutrients were added to both fresh water and waste water mediums in this study in order to compare algae growth in both mediums. Waste water characterization showed that waste water usually has nitrate and phosphate in different concentrations. Therefore, more work is needed to study algae growth in treated waste water with altering the concentrations of the nutrients added in order to save chemicals and reach the optimum growth.

2. LEDs use in algae growth could be extended to study "Smart Photokinetics." This allows the use of real-time analysis to change the lighting profile to make it suitable for an algae batch at a particular stage of growth. Light sensors in the photobioreactor measure the instantaneous turbidity and send the data to a microcontroller. The controller logs data and compares turbidity at each time period to previous data. A decision algorithm determines when growth has reached a new phase and implements a more effective lighting profile.
3. Extend the kinetics model developed in this work to study lag and exponential phase in order to determine the specific growth rate, which is important for the design of the Photobioreactor.

4. The present work was successful in studying the effect of nitrogen deprivation on microalgae lipid production. More work needs to be done to study the phosphate and iron deprivations as another methods of lipid triggering to obtain the optimum conditions for microalgae growth.

5. Chlorella vulgaris biodiesel produced in this study by in-situ transestrification was not easy to be handled. It was difficult to measure its volume experimentally. That may be due to high specific gravity of vulgaris biodiesel (1.24) obtained in this study. More work need to be constructed to overcome this obstacle such as using purification method to separate any solids from the biodiesel produced.

6. Large-scale biodiesel production through the one-step (in-situ transestrification) process needs to be investigated in order to enhance blending process and achieve better economic bio-jet fuel production.
7 REFERENCES


Ramirez-Verduzco, L. F., Javier Esteban Rodriguez-Rodriguez, Alicia del Rayo Jaramillo-Jacob, “Predicting Cetane number, kinematic viscosity, density and higher heating value of biodiesel from its fatty acid methyl ester composition”, Fuel, 2011, volume 91, pages 102-111.

Ramsey, J. P., "Design of Experiments", Class Notes, Math 840, UNH, 2004


Scarsella, M., Belotti, G., De Filippis, P., Bravi, M., “Study on the optimal growing conditions of Chlorella vulgaris in bubble column photobioreactors”, Dept. of Chemical Engineering Materials Environment, Sapienza University of Roma Via Eudossiana 18, I-00184 Roma, Italy.


Wilson, R., G. Salama, I. Farag, "Microalgae Growth in Qatar for CO2 Capture and Biodiesel Feedstock Production,” Global Journal of Researches in Engineering (GJRE), Chemical Engineering, Volume 12 Issue 1 Version 1.0 Year 2012, Online ISSN: 2249-4596 & Print ISSN: 0975-5861


http://www.jofamericanscience.org/journals/amsci/am0802/055_8150am0802_392_398.pdf
APPENDICES
PICHURES OF THE 2L PBR AND THE 80L PBR SYSTEMS

A. 2L PBR

- Algae culture in the 2L PBR

Figure I.1 Four liter fish tank reactor used in Algae Growth. The tank is divided vertically into two identical 2L reactors. Algae cultures in the two 2L PBR are exposed to the same lights and air bubbling. First use of the Figure in the thesis: Chapter 3, Section 3.4.1

- Light Sources

(a) Red-Blue LEDs Panels  (b) Red LEDs Panel  (c) Fluorescent light

Figure I.2: Different light sources: (a) Red-Blue LEDs panels, (b) Red LEDs panels and (c) Fluorescent light First use of the Figure in the thesis: Chapter 3, Section 3.5
- Air supply

Figure I.3 Air stone
First use of the Figure in the thesis: Chapter 3, Section 3.4.3

B. 80 L PBR

Figure I.4: Algae culture in the 80 L PBR
First use of the Figure in the thesis: Chapter 3, Section 3.4.2

Figure I.5: The harvested algae culture in four 5-gallon carboys
First use of the Figure in the thesis: Chapter 3, Section 3.4.2

LEDs Jacket construction (Eltringham, 2013)

This LEDs jacket consists of

a. 12V Flexible waterproof LED strips available in red and blue colors. AC/DC adapters powered each strip and were connected to a single mains power switch to allow the LED array to be easily turned off.
b. Reflective Mylar sheet was used as a base to reflect the unabsorbed light back to the algae solution to improve efficiency.

c. Wide Velcro was used for an adjustable fit.

(a) Blue and Red LED strips  (b) Reflective Mylar sheet  (c) Wide Velcro

Figure 1.6: LEDs jacket materials: (a) Blue and Red LED strips, (b) Reflective Mylar sheet, (c) Wide Velcro
First use of the Figure in the thesis: Chapter 3, Section 3.7.2

Figure 1.7: LEDs Jacket
First use of the Figure in the thesis: Chapter 3, Section 3.7.2
A. HP PEAK 96 SETTING

1. Run peak96 on the computer connected to HP integrator; make sure the integrator is turned on before running Peak96.

2. From peak 96, go to Data Acquisition, Set up PC, Generate New and type the following:
   - At Data Path \peak\folder name
   - At Export Path \peak\folder name
   - At Auto Export
     - Report to ASCII Y
     - Data to ChemStation Y
     - Data to ASCII Y

   The data transferred from the integrator to Peak96 will be saved in this folder stated in the following directory: \c:\mycomputer\peak96\folder name. The data will be saved as “asc” format.

3. Go to Utilities, Files, Export. From Text, choose the file and hit Enter. From Signal Data, choose the file and hit Enter.

4. Go to Data Acquisition, then Set up Integrator as follows:

   4.1 Integration plot type: F

   Presentation plot: NO

   4.2 Data Storage options

   - Store Signal data (Y/N): YES
   - Local run time data storage (Y/N): YES
   - Keep runtime data storage (Y/N): YES
   - Store processed peaks (Y/N): YES

   4.3 Report options

   - Report uncalibrated peaks (Y/N): YES

   4.4 Post-run list options
Large font: YES
Store post-run report (Y/N): YES
List run parameters (Y/N): YES
List remote method (Y/N): YES

4.5 Save current method: RACHEL.MET

4.6 Select old method: Select RACHEL.MET, then hit Enter.

4.7 Down load method: select RACHEL.MET

The following message will show up “Downloading method RACHEL.MET to the integrator”

5. Inject the sample in the GC, then go to Data Acquisition, select RUN, then select START RUN and hit Enter.

The data will be saved as document file “txt” format, which involves the same data printed out from the integrator and as an “asc” format.

B. STEPS TO CONVERT “asc” FORMAT TO “csv” FORMAT

The “asc” should be converted to “csv” format in order to create a chromatogram using Excel. The next steps should be followed to construct peak chromatogram such as figures 4.30, 4.31, 4.32 and 4.36.

1. Run Excel, go to File, select Open, choose “All files” from File name, then choose the “asc” file and hit open.

2. Text Import Wizard steps 1-3 window will appear.

   First step: select Delimited, then hit next
   Second step: select Comma from Delimiters and hit next
   Third step: select General from Column data format and hit Finish

   Two numeric columns will appear in the Excel file: Column A and B

3. To convert column A to peak height (arbitrary units), use the following equation:

202
Peak height = A + (the number in A1 in negative)
To convert column B to time (minutes), use the following equation:
Time = 9.006 * B / (275*10240)

4. Insert a scatter chart with smooth lines, Peak height as Y-axis and Time as X-axis.
A. ANOVA ANALYSIS STEPS

1. Run JMP and add the data in four columns; Algae species, light source, water source and lipid production (mg/L Solution-day)

2. Select the menu option (Fit Model) from Analyze.

3. High light algae species, light source and water source from selected columns and press Add.


5. High light water source from Construct Model Effects, High light algae species from Selected Columns and press (Cross).

6. High light lipid production from Selected Columns and press Y.

7. Select the menu option (Full Factorial) from (Macros).

8. Select the menu option (Effect Screening) from (Emphasis).

9. Press (Run).

10. From the red arrow, select the menu option (Script) and select (Save Script to Data Table) from the sub menu.

11. Click the red arrow to select the menu option (Factor profiling). Then, select (Interaction plots) from the sub menu.
B. STUDENT’S TEST STEPS

1. Run JMP; select the menu option (Fit Y by X) from Analyze.

2. Highlight (light source and water source from Selected Columns and press X, Factor.

3. Highlight lipid production from Selected Columns and press Y, Response.

4. Press OK.

5. Click the red arrow to select the menu option (Compare Means) then select the desired multiple comparison technique (Student’s t test) from the sub menu.
<table>
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<th>Columns (4/1)</th>
<th>A</th>
<th>Algae species</th>
<th>Light Source</th>
<th>Water Source</th>
<th>Lipid production (mg)</th>
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<td>22</td>
<td>Chlorella vulgari</td>
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<td>28</td>
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<td></td>
<td>30</td>
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<td>mix</td>
<td>Waste water</td>
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</tr>
</tbody>
</table>

Distribution of Y for each X. Modeling types determine analysis.
# APPENDIX IV

## A. EFFECT OF MEDIUM AND LIGHT SOURCES ON CHLORELLA VULGARIS BIOMASS PRODUCTION (mg dry algae/L Solution-day)

First use of this Appendix in the thesis: Chapter 4 Section 4.1.4

<table>
<thead>
<tr>
<th>Replication number</th>
<th>fresh water Red LEDs</th>
<th>waste water Red LEDs</th>
<th>fresh water Red-Blue LEDs</th>
<th>waste water Red-Blue LEDs</th>
<th>fresh water Fluorescent</th>
<th>waste water Fluorescent</th>
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<tbody>
<tr>
<td>1</td>
<td>108.3</td>
<td>69.4</td>
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<td>90.6</td>
<td>88.3</td>
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<td>260</td>
<td>152.1</td>
<td>245.7</td>
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<td>111.4</td>
<td>128.1</td>
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<td>71.9</td>
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</table>

## B. EFFECT OF MEDIUM AND LIGHT SOURCES ON CHLORELLA C2 BIOMASS PRODUCTION (mg dry algae/L Solution-day)

<table>
<thead>
<tr>
<th>Replication number</th>
<th>fresh water Red LEDs</th>
<th>waste water Red LEDs</th>
<th>fresh water Red-Blue LEDs</th>
<th>waste water Red-Blue LEDs</th>
<th>fresh water Fluorescent</th>
<th>waste water Fluorescent</th>
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</thead>
<tbody>
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<td>84.4</td>
<td>148.9</td>
<td>117.9</td>
<td>59.9</td>
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</table>

## C. EFFECT OF MEDIUM AND LIGHT SOURCES ON CHLORELLA VULGARIS LIPID YIELD (mg lipid/100 g dry algae)

<table>
<thead>
<tr>
<th>Replication number</th>
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<th>fresh water Red-Blue LEDs</th>
<th>waste water Red-Blue LEDs</th>
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</thead>
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<td>5.6</td>
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<td>3.8</td>
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</tbody>
</table>
D. EFFECT OF MEDIUM AND LIGHT SOURCES ON CHLORELLA C2 LIPID YIELD (mg lipid/100 g dry algae)

<table>
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<tr>
<th>Replication number</th>
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<th>waste water Red LEDs</th>
<th>fresh water Red-Blue LEDs</th>
<th>waste water Red-Blue LEDs</th>
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<td>1.43</td>
<td>2.69</td>
<td>1.89</td>
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<td>3</td>
<td>2.6</td>
<td>1.6</td>
<td>2.38</td>
<td>1.62</td>
<td>2.48</td>
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</table>

E. EFFECT OF MEDIUM AND LIGHT SOURCES ON CHLORELLA VULGARS LIPID PRODUCTION (mg lipid/L Solution-day)

<table>
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<th>fresh water Red-Blue LEDs</th>
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<td>4.7</td>
<td>8.5</td>
<td>2.6</td>
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<td>7.9</td>
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</tr>
<tr>
<td>3</td>
<td>4.6</td>
<td>3.3</td>
<td>7.2</td>
<td>4.2</td>
<td>2.7</td>
<td>5.8</td>
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</table>

F. EFFECT OF MEDIUM AND LIGHT SOURCES ON CHLORELLA C2 LIPID PRODUCTION (mg lipid/ L Solution-day)

<table>
<thead>
<tr>
<th>Replication number</th>
<th>fresh water Red LEDs</th>
<th>waste water Red LEDs</th>
<th>fresh water Red-Blue LEDs</th>
<th>waste water Red-Blue LEDs</th>
<th>fresh water Fluorescent</th>
<th>waste water Fluorescent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.29</td>
<td>6.7</td>
<td>3.52</td>
<td>3.98</td>
<td>0.38</td>
<td>0.29</td>
</tr>
<tr>
<td>2</td>
<td>1.69</td>
<td>0.57</td>
<td>0.57</td>
<td>0.61</td>
<td>1.63</td>
<td>0.83</td>
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<tr>
<td>3</td>
<td>3.25</td>
<td>1.35</td>
<td>3.54</td>
<td>1.91</td>
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G. EFFECT OF MEDIUM SOURCE ON LIPID CONTENT IN SCALED-UP PBR.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Algae Used</th>
<th>Medium Source</th>
<th>Light Source</th>
<th>Lipid Yield, g lipid/100 g algae</th>
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</thead>
<tbody>
<tr>
<td>A1</td>
<td>Chlorella vulgaris</td>
<td>Fresh water</td>
<td>Fluorescent light</td>
<td>4.5</td>
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<tr>
<td>A1</td>
<td>Chlorella vulgaris</td>
<td>Fresh water</td>
<td>Fluorescent light</td>
<td>4.3</td>
</tr>
<tr>
<td>A3</td>
<td>Chlorella vulgaris</td>
<td>Fresh water</td>
<td>Fluorescent light</td>
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H. EFFECT OF CHLORELLA VULGARIS NITROGEN DEPRIVATION ON FAME CONCENTRATIONS

<table>
<thead>
<tr>
<th>KNO₃g/l</th>
<th>FAME concentration (mg FAME/g of dry algae)</th>
<th>C16:1</th>
<th>C16:0</th>
<th>C18:1,2,3</th>
<th>C18:0</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.525</td>
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<td>2.7</td>
<td>8.59</td>
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<td>2.69</td>
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<tr>
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<tr>
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<td>1.92</td>
<td>16.1</td>
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</tbody>
</table>

I. EFFECT OF CHLORELLA C2 NITROGEN DEPRIVATION ON FAME CONCENTRATIONS

<table>
<thead>
<tr>
<th>KNO₃g/l</th>
<th>FAME concentration (mg FAME/g of dry algae)</th>
<th>C16:1</th>
<th>C16:0</th>
<th>C18:1,2,3</th>
<th>C18:0</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.525</td>
<td>0.2</td>
<td>0.08</td>
<td>1.38</td>
<td>3.38</td>
<td>1.13</td>
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<tr>
<td>0.2625</td>
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<td>2.85</td>
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