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Biodiesel Production from Mixed Culture Algae Via a Wet Lipid Extraction Procedure

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BIODIESEL PRODUCTION FROM MIXED CULTURE ALGAE VIA A WET LIPID EXTRACTION PROCEDURE

by

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ABSTRACT

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Via a Wet Lipid Extraction Procedure

by

Ashik Sathish, Master of Science

Utah State University, 2012

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With world crude oil reserves decreasing and energy prices continually increasing, interest in developing renewable alternatives to petroleum-based liquid fuels has increased. An alternative that has received consideration is the growth and harvest of microalgae for the production of biodiesel via extraction of the microalgal oil or lipids. However, costs related to the growth, harvesting and dewatering, and processing of algal biomass have limited commercial scale production of algal biodiesel. Coupling wastewater remediation to microalgal growth can lower costs associated with large scale growth of microalgae. Microalgae are capable of assimilating inorganic nitrogen and phosphorous from wastewater into the biomass. By harvesting the microalgal biomass these nutrients can be removed, thus remediating the wastewater. Standard methods of oil extraction require drying the harvested biomass, adding significant energetic cost to processing the algal biomass. Extracting algal lipids from wet microalgal biomass using traditional methods leads to drastic reductions in extraction efficiency, driving up processing costs. A wet lipid extraction procedure was developed that was capable of extracting 79% of the transesterifiable lipids from wet algal biomass (16% solids) without the use of organic solvents while using relatively mild conditions (90 °C and ambient pressures). Ultimately 77% of the extracted lipids were collected for biodiesel production. Furthermore, the

procedure was capable of precipitating chlorophyll, allowing for the collection of algal lipids independently of chlorophyll. The capability of this procedure to extract lipids from wet algal biomass, to reduce chlorophyll contamination of the algal oil, and to generate feedstock material for the production of additional bio-products provides the basis for reducing scale-up costs associated with the production of algal biofuels and bioproducts.

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PUBLIC ABSTRACT

Development of renewable sources of energy has received significant interest due to the rising costs of energy and the environmental impact of using fossil fuels. Biodiesel production from renewable sources of oil has shown promise of helping to replace or reduce dependence on petroleum based diesel thereby reducing demand for crude oil. Microalgae have been considered as a strong candidate for the production of large quantities of renewable oil for biodiesel production.

Microalgae are single cell photosynthetic organisms that posses the capability to produce renewable oil at rates much faster than land based plants and crops. In addition, microalgae can be grown in non-arable land, use low quality water or wastewater, and do not require significant maintenance making algal biomass simpler to generate and help in avoiding the food versus fuel debate. However, the current cost of processing algae has prevented commercial production of algae biodiesel. Several hurdles exist that contribute to the production cost, some of which include: (1) the need to dry algal biomass prior to lipid extraction when using traditional methods of oil extraction, (2) large volumes of organic solvents commonly required to extract the algal oils, and (3) purification costs associated with generating usable biodiesel.

This research focused on developing a method of processing algal biomass to help directly address these hurdles. The wet lipid extraction procedure developed is capable of extracting oil from algal biomass with no drying, reduces the demand for organic solvents, and removes or reduces chlorophyll contamination from the produced biodiesel potentially reducing purification requirements. Additionally, the developed procedure produces several additional streams that can be utilized as feedstock material for the production of additional algae based bioproducts. Such advances in algal processing technology can aid in reducing the cost of producing renewable microalgae based biofuels and bioproducts.

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Ashik Sathish

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CHAPTER 1

INTRODUCTION AND NEED FOR STUDY

1. Need for renewable energy

Energy demand worldwide continues to increase at a rapid pace with developing countries, such as China and India's, energy consumption rates rising at 2 to 3 times the global average (Ahmad et al., 2011; U.S. Energy Information Administration, 2011a). Crude oil reserves are being depleted (Abdullah et al., 2007) at a rate of approximately 85-90 million barrels of oil per day and predicted to increase as shown in Figure 1(U.S. Energy Information Administration, 2011a). With worldwide proven oil reserves estimated at 1.3 trillion barrels of oil (U.S. Energy Information Administration, 2011a), it is possible that crude oil will be depleted within the next 50 years (Abdullah et al., 2007).

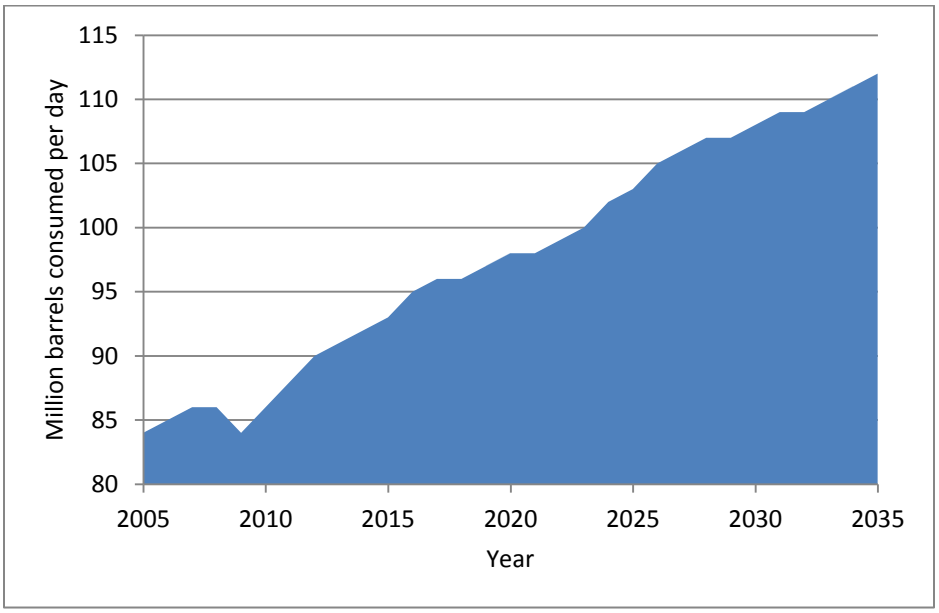

Figure 1. Projected global crude oil consumption.

The US currently consumes approximately 20% of the crude oil consumed globally (U.S. Energy Information Administration, 2011a) and petroleum accounts for 37% of the total energy flow within the US (U.S. Energy Information Administration, 2011b). Of the energy generated

from crude oil, or petroleum, 72% is consumed for transportation purposes (U.S. Energy Information Administration, 2011b). A majority of the energy used within the transportation sector originates from petroleum, which constitutes 94% of the energy consumed within that sector. Heavy dependence on petroleum based fuels, is not sustainable due to increasing fuel costs, diminishing crude oil reserves, and the environmental impact of fossil fuel usage (Chisti, 2007; Pienkos and Darzins, 2009; Demirbas and Fatih Demirbas, 2011).

Alternative sources of energy will need to be research and developed within the next 50 years as crude oil resources become scarce. Continued research will facilitate the development and implementation of renewable fuels to help lessen the US's and world's dependence on fossil fuels as petroleum based fuels become depleted.

2. Microalgae as a source of renewable oil for biodiesel production

The US DOE supported Aquatic Species Program (1978-1996) conducted research to utilize microalgae as a source of oil for the production of renewable fuels with a focus on biodiesel (Sheehan et al., 1998). However, due to the high cost of biodiesel production from algal biomass and DOE budget constraints at the time, the Aquatic Species Program was terminated (Sheehan and others, 1998).

Recent increases in the price crude oil, environmental concerns, and the US's significant dependence on foreign oil have revived interest in making use of microalgae to produce renewable liquid fuels and other bioproducts (U.S. DOE, 2010). Interest increased after the enactment of The Energy Independence and Security Act (EISA) of 2007 that mandated by 2022, 36 billion gallons of renewable fuel, including cellulosic and biomass derived diesel, should be a portion of the transportation fuel consumed in the US (U.S. DOE, 2010).

Several reasons have attracted interest for the use of microalgae over other energy crops or animal fats including: (1) microalgae have higher growth rates than terrestrial crops; (2) can be grown in non-arable or marginal lands with various qualities of water; (3) require little

maintenance; (4) generate high concentrations of intracellular lipids; (5) provide a means to recycle CO_2; and (6) do not require diverting food resources to energy production (Chisti, 2007; Gouveia and Oliveira, 2008; Azócar et al., 2010; Huang et al., 2010; Mata et al., 2010; U.S. DOE, 2010; Wijffels et al., 2010). Table 1 presents data illustrating the high productivity of microalgae compared to terrestrial oil crops. These properties and capabilities of microalgae make them an appealing means for the production of renewable oil for biodiesel production.

Table 1. Comparison of oil productivity of various oil crops for biodiesel production (Gouveia and Oliveira, 2008).

Crop	Oil Yield (L ha^{-1})
Corn	172
Soybean	446
Canola	1,190
Jatropha	1,892
Coconut	2,689
Palm	5,950
Microalgae [a]	58,700
Microalgae [b]	136,900

[a] 30% oil (by wt) in biomass
[b] 70% oil (by wt) in biomass

Because of these capabilities, microalgae can be used for a variety of applications including wastewater remediation, carbon sequestration from flue gases (coal fired power plants), and removal of heavy metals from industrial wastewaters while generating biomass for biofuel and bioproduct production (Pokoo-Aikins et al., 2009; Harun et al., 2010; Rawat et al., 2011).

Although microalgae are candidates as a renewable source of oil and the concept of algal biodiesel production has been proven and well studied, there are currently no known commercial scale algal biodiesel production facilities (Lardon et al., 2009). This is due to the high cost associated with the production of algal biodiesel, which makes biodiesel uncompetitive with petroleum based diesel.

One of the major hurdles in reducing the cost of algal biodiesel is the need to dewater the algal biomass after harvesting the algae from the growth medium (Lardon et al., 2009; Levine et

al., 2010; Sander and Murthy, 2010). The presence of water with the harvested biomass inhibits organic solvent based extraction of lipids from algal biomass (U.S. DOE, 2010). Additionally, the direct transesterification of algal oils by *in situ* transesterification is also inhibited by the presence of water (Liu et al., 2006). Without drying the algal biomass to at least 90% solids, the efficiency of either solvent based lipid extraction or *in situ* transesterification will be reduced leading to higher processing costs (Lardon et al., 2009). However, drying large quantities of algal biomass requires a considerable amount of energy to remove water from the biomass which drives up the cost of algal biodiesel production (Sturm and Lamer, 2011).

Methods are available to extract and/or convert algal lipids to biodiesel from wet algal biomass, but many require the use of super or sub critical fluids (Demirbas, 2006; Levine et al., 2010; Halim et al., 2011). These processes require significant energy input and are difficult to scale up for the production of large amounts of biodiesel (Halim et al., 2011). Therefore, there is a need for the development of low cost processes for the extraction of algal oil from wet biomass to aid in generating economically viable production processes for algal biodiesel.

3. Logan Lagoons Project

The city of Logan, UT Environmental Department operates and maintains a 460 acre open lagoon wastewater treatment plant (WWTP) to treat municipal wastewater generated within the city of Logan and surrounding regions. The Logan Lagoons wastewater treatment system is capable of primary and secondary treatment (solids, biological oxygen demand, and pathogen removal). However, it is not designed for tertiary treatment of the wastewater (removal of inorganic nutrients) (Griffiths, 2009).

With State regulations requiring reductions in the amount of nitrogen and phosphorous being discharged from the Logan lagoons WWTP, the city will be required to modify the current system. This is generally accomplished with the addition of a physical or chemical treatment system, which is a costly addition (Sturm and Lamer, 2011). Such a costly modification can be

avoided by utilizing microalgae that naturally grow within the Logan lagoons WWTP. Microalgae take up nitrogen and phosphorous as nutrients as they grow. Therefore, the implementation of a harvesting system for algal biomass would not only provide a means to sustainably remove these nutrients, but would also provide a supply of renewable feedstock for the production of algal biodiesel.

The opportunity to couple wastewater treatment with microalgal growth provides a means to reduce production cost due to the availability of free nutrients, sunlight, and atmospheric carbon dioxide. Current estimates place the algal production potential of the Logan Lagoons around 13,000 kg/day of dry algal biomass, with enough lipids to produce approximately 120,000 gallons of biodiesel per 300 day year (Griffiths, 2009). The energy generated from this system can be used to supplement the City of Logan Environmental Department's energy needs helping to reduce their dependence on imported fuels and energy.

The potential cost savings from coupling wastewater remediation with algal biomass generation and the use of a wet lipid extraction procedure can help reduce the cost of algal biodiesel production. Such a system can sustainably remove nutrients by providing a means to capture and utilize inorganic nitrogen and phosphorous removing it from the wastewater and in return the biomass can be processed to generate energy via both liquid fuels and methane gas.

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CHAPTER 2

LARGE SCALE PRODUCTION OF MICROALGAL BIOMASS AND METHODS FOR THE EXTRACTION AND CONVERSION OF MICROALGAL OIL TO BIODIESEL

1. Introduction

In 2010 petroleum was the primary source of energy in the US, accounting for 37% of the total energy flow within the country. Additionally, 71% of the energy generated from petroleum was accounted for within the transportation sector, constituting 94% of the energy used in that sector (U.S. Energy Information Administration, 2011). Approximately 13 million barrels per day of transportation fuel was consumed in 2009, including 2 million barrels a day of on highway diesel (U.S. Energy Information Administration, 2011). Such dependence on petroleum based fuels is not sustainable due to increasing fuel costs, steady depletion of world crude oil reserves, and the environmental consequences associated with the use of fossil fuels (Chisti, 2007; Lardon et al., 2009; Demirbas and Fatih Demirbas, 2011). These and other problems related to the use of fossil fuels have led to interest in finding alternative sources of energy.

Considerable efforts are being made to develop commercially viable sources of renewable transportation fuels to lessen world dependence on crude oil. Of the various sources of renewable liquid fuels, production of biodiesel from biological lipids, or oil, shows potential.

A common lipid sources is plant oils, of which soybean, rapeseed, and sunflower oils are the most prevalent in the US and Europe (Azócar et al., 2010). Although these types of oils are produced in high quantities, on the order of 88 million tons of vegetable oil in 2000 (Demirbas, 2009), there are several constraints associated with the use of these edible oils for biodiesel production. One concern is that food resources and land would be diverted to energy production, potentially driving up food costs, considered the “food vs. fuel” debate (Schenk et al., 2008;

Azócar et al., 2010; Mata et al., 2010; Vyas et al., 2010). Due to this concern, attention has turned to lipids derived from non-edible plants such as rubber seeds, Jatropha, tobacco, and castor oils (Demirbas, 2009; Azócar et al., 2010). These oils are generally cheaper and do not interfere with food production resources (Azócar et al., 2010).

Waste oils are another potential source of lipids for biodiesel production. Waste oils refer to used cooking oils as well as rendered animal fats (Kulkarni and Dalai, 2006). Lipid sources such as these are cheap compared to pure vegetable oils and are readily available as a feedstock (Kulkarni and Dalai, 2006; Canakci, 2007) . High FFA content can interfere with the reaction that converts lipids to biodiesel.

Typically the conversion of oils to biodiesel is performed using a homogenous base catalyst. Base catalyzed reactions are quicker and require less energy compared to acid catalyzed processes (Freedman et al., 1986; Ma and Hanna, 1999; Fukuda et al., 2001; Meher et al., 2006). However, the use of base catalysts leads to soap formation when FFAs are present via the saponification reaction, which reduces reaction efficiency and complicates downstream separation and purification of the generated biodiesel (Leung et al., 2010). One alternative is to use acid catalysts when FFAs are present, due to its ability to catalyze the conversion of FFAs to alkyl esters by esterification (Canakci and Van Gerpen, 2001; Lotero et al., 2005; Van Gerpen, 2005). These topics are discussed in more detail in Section 5.

Although plant and animal oils can provide a source of oil for biodiesel, current land requirements for growth of terrestrial plant material for fuel production are substantial. For example, 13% and 15% of US and European farmland, respectively, would be needed to generate enough vegetable oil for biodiesel to displace 5% of the petroleum based diesel consumed in these regions (Azócar et al., 2010). Such large land requirements make replacing petroleum diesel with biodiesel solely from plant oil based biodiesel unfeasible and puts food production at risk.

Another source of lipids that has shown promise is microalgae. Table 2 illustrates higher oil productivity of microalgae compared to typical biodiesel crops. Besides being more efficient

in producing oil, microalgae also possess qualities that make them viable as a source of lipids for large scale biodiesel production. These qualities include the fact that microalgae: (1) do not shift food resources to energy production, (2) have higher growth rates than land based crops, (3) can be grown in non-arable land using various sources and qualities of water, (4) require little maintenance, (5) and can produce high concentrations of intracellular lipids (Chisti, 2007; Gouveia and Oliveira, 2008; Azócar et al., 2010; Huang et al., 2010; Mata et al., 2010; Wijffels et al., 2010). Characteristics such as these make microalgae a promising source of lipids for biodiesel production on an appreciable scale.

Table 2. Comparison of oil yields from typical energy crops versus microalgae. Adapted from Mata et al. (2010).

Plant Source:	**Oil yield (L oil/ha yr)**	**Land Use (m^2 year/kg biodiesel)**	**Biodiesel Productivity (kg biodiesel/ha yr)**
Corn/Maize (*Zea mays* L.)	172	66	152
Hemp (*Cannabis sativa* L.)	363	31	321
Soybean (*Glycine max* L.)	636	18	562
Jatropha (*Jatropha curcas* L.)	741	15	656
Camelina (*Camelina sativa* L.)	915	12	809
Canola/Rapeseed (*Brassica napus* L.)	974	12	862
Sunflower (*Helianthus annuus* L.)	1,070	11	946
Castor (*Ricinus communis*)	1,307	9	1,156
Palm oil (*Elaeis guineensis*)	5,366	2	4,747
Microalgae (low oil content)	58,700	0.2	51,927
Microalgae (medium oil content)	97,800	0.1	86,515
Microalgae (high oil content)	136,900	0.1	121,104

2. Large-Scale Algae Growth Systems:

A number of technologies exist for the growth and collection of microalgae, with a majority designed as either closed or open growth systems. Open systems can take a number of different forms such as raceway ponds, circular ponds, tanks, and shallow ponds (Molina Grima et al., 2003; Chisti, 2007; Harun et al., 2010). The most common forms are shallow raceway ponds with a source of mixing, such as a paddle wheel, to allow for light penetration, gas diffusion, and nutrient distribution (Christenson and Sims, 2011; Gallagher, 2011).

2.1. Open systems

Open systems are cheaper to maintain and construct due to their simple operation and basic design (Harun et al., 2010; Stephenson et al., 2010; Christenson and Sims, 2011; Gallagher, 2011). However, the fact that they are open systems is their disadvantage as well. Due to open systems being exposed to the atmosphere, conditions within the ponds depends on the surrounding environment. Temperature, sunlight, pH, and evaporation may all be environmentally dictated. Without control over these factors shifts in species may be unavoidable, due to natural selection within the ponds and/or outside contamination, leading to the loss of single strain cultures (Chisti, 2007; Harun et al., 2010; Stephenson et al., 2010; Christenson and Sims, 2011).

2.2. Closed systems

Lack of control over environmental conditions within algae growth systems has pushed efforts to build and design effective and economical closed systems for microalgal growth. Closed systems, such as tubular photobioreactors, allow for conditions to be maintained close to the desired set-points without significant deviation (Mata et al., 2010; Dye et al., 2011). Such control allows for the growth of algae with minimal shifts in species or contamination from external organisms, essentially the ability to grow single strains of algae (Chisti, 2007; Harun et al., 2010; Christenson and Sims, 2011). With the choice of which microalgal strains to culture in larger closed systems, it is possible to cultivate specific species that show tendencies to

accumulate high percentages of oil leading to higher lipid productivities (Stephenson et al., 2010).

Major drawbacks with closed systems include the inability to remove oxygen, which builds up within the system as a result of photosynthesis, difficulties in controlling temperature, energy costs required for mixing and maintenance of static conditions, and the capital cost associated with building such systems (Scott et al., 2010; Christenson and Sims, 2011).

2.3. For biodiesel production and wastewater remediation

Specifically for biodiesel production, open systems are the most feasible due to the higher capital and operating costs associated with closed systems. For algae biodiesel to be a feasible source of fuel, a requirement is the production costs must be lower than the cost of producing petroleum diesel. Open systems for microalgal growth are currently the most viable option due to its low capital cost as well as lower operation and maintenance costs (Huang et al., 2010).

One of the most promising processes for microalgal biomass generation is the combination of wastewater treatment with microalgal growth using open systems (Chinnasamy et al., 2010; Christenson and Sims, 2011; Pittman et al., 2011). Such systems have the ability to generate, not only algal biomass, but also remediate wastewater by taking up excess nutrients from the water. Treating wastewater using microalgae is also a method of recovering nutrients, such as nitrogen and phosphorous, in a sustainable manner. The nutrients present in wastewater replaces the need for the addition of nitrogen and phosphorous for algal growth removing the need for energy input into fertilizer production for algal growth (Clarens et al., 2010).

3. Collection and Dewatering of Algal Biomass

With algal cell sizes ranging from 2 to 20 μm, coupled with the fact that they are generally grown in suspended form, leads to difficulties in harvesting and concentrating algal biomass (Lardon et al., 2009). A number of processes exist for the collection of algal biomass including those based on chemical, mechanical, physical, and biological methods (Molina Grima et al., 2003; Uduman et al., 2010; Christenson and Sims, 2011). It can be assumed that 20-30% of

the cost of producing algal biomass can be attributed to the harvesting step (Molina Grima et al., 2003). In addition, Lardon et al. found that dewatering and drying of algae accounts for approximately 80 - 85% of the total energy used to generate biodiesel from algae (Lardon et al., 2009). Many biodiesel producing processes require the drying of the algal biomass prior to extraction and conversion of the oil to biodiesel (Ehimen et al., 2010; Levine et al., 2010; Halim et al., 2011). Based on this information any harvesting method used must be able to collect algal biomass with maximum dewatering and minimal energy usage to help reduce biodiesel production costs.

3.1. Centrifugation

Centrifugation currently remains the most commonly used method for the collection of algae (Harun et al., 2010). Although centrifugation of algae is effective, with recoveries over 90% with approximately 22% solids content (Christenson and Sims, 2011), the energy consumption associated with its use is considered high (Lardon et al., 2009). Centrifugation can be justified if high value products are generated, but for production of biodiesel, more energy efficient methods must be used (Molina Grima et al., 2003; Pienkos and Darzins, 2009; Uduman et al., 2010).

3.2. Filtration

Several types of filtration systems exist for algae collection including dead end, tangential flow, pressure filtration, and microfiltration (Harun et al., 2010). In spite of filtration's simplicity, there are several costly drawbacks to using filtration. Because filtration is based on the exclusion of particles based on size via a membrane, it suffers from continuous fouling problems and requires constant maintenance (Mata et al., 2010; Uduman et al., 2010). Filtration has been cited as being relatively slow compared to other harvesting methods, which is disadvantageous for large scale operations (Molina Grima et al., 2003). The regular need to replace filter membranes can become costly, especially when using expensive membranes (Harun et al., 2010;

Christenson and Sims, 2011). The most probable use of filtration will be in series with another dewatering system (Harun et al., 2010).

3.3. Gravity sedimentation flocculation

Gravity settling or sedimentation of algal biomass has been viewed as a low cost option to concentrate algal biomass (Pienkos and Darzins, 2009). However, sedimentation methods that only use gravity are fairly inefficient and costly due to the small cell sizes of microalgae and slow settling rates (Schenk et al., 2008). Sedimentation can be accelerated with the use of flocculants, which promote the aggregation of algal cells, forming larger particles increasing the rate of settling (Molina Grima et al., 2003; Mata et al., 2010). Flocculation can be induced by increasing the pH of the medium (Molina Grima et al., 2003), varying the environment to cause bio-flocculation or auto-flocculation (spontaneous formation of algal floccs) (Uduman et al., 2010), addition of chemicals such as aluminum sulfate, or the addition of organic and/or inorganic polymers (Molina Grima et al., 2003; Pienkos and Darzins, 2009; Harun et al., 2010). Once flocculated the larger particles sediment much more rapidly increasing the collection efficiency (Christenson and Sims, 2011). Subsequent collection of these larger particles, or algal floccs, can be accomplished by centrifugation, filtration, of dissolved air flotation methods (Harun et al., 2010; Uduman et al., 2010; Christenson and Sims, 2011). Drawbacks involve the cost associated with the addition of flocculants (Pienkos and Darzins, 2009). Also addition of certain compounds may cause downstream purification problems, especially with addition of metal salts (Molina Grima et al., 2003).

3.4. Combined processes for dewatering

Each of these methods when analyzed individually have certain advantages, drawbacks, associated capital and operation costs, and may be ineffective used by themselves. It is possible to combine flocculation with centrifugation, or filtration with centrifugation, for example to help reduce the cost of harvesting large quantities of algal biomass. Such combinations may reduce the

energy demand of harvesting algae as well as increase the level of dewatering. Table 3 presents energy consumption of algae collection and drying methods, while Table 4 summarizes characteristics of some of the methods discussed.

Table 3. Heat energy required to dewater algal biomass to the specified moisture content and generate 1 kg biodiesel. D.C.C. refers to dry cell concentration.

Unit Operation(s) Used:	Moisture Content:	Energy: (MJ)	Source:
Filter Press and natural gas dryer:	9 wt% moisture	120.31	[(Sander and Murthy, 2010)]
Centrifugation and natural gas dryer:	9 wt% moisture	237.03	[(Sander and Murthy, 2010)]
Chemical Flocculation and belt Press:	10 wt% moisture	81.8	[(Lardon et al., 2009)]
Flocculation and centrifugation:	200 kg/m^3 D.C.C.	2.5	[(Stephenson et al., 2010)]
Flocculation, centrifugation, and mechanical and thermal dryer:	< 15% moisture	238	[(Xu et al., 2011)]
Chemical flocculation and belt press:	10 wt% moisture	319	[(Razon and Tan, 2011)]

Table 4. Comparison of mechanical harvesting methods for algal biomass. Adapted from Christenson et al.

Method	Solids Concentration	Recovery	Major limitations
Centrifugation	12-22%	>90%	Energy intensive, high cost
Tangential Filtration	5-27%	70-90%	Membrane fouling, high cost
Gravity sedimentation	0.5-3%	10-90%	Slow, unreliable
Dissolved air floatation	3-6%	50-90%	Flocculants usually required

4. Methods of oil extraction

In order to produce biodiesel from algal biomass cellular lipids must be extracted from the cell and collected. This requires the disruption or rupture of the algal cell and can be achieved using a variety of methods including mechanical and chemical disruption, solvent extraction, supercritical fluid extraction, and combinations and/or variations of these techniques (Lee et al., 2010; U.S. DOE, 2010; D'Oca et al., 2011; Mercer and Armenta, 2011).

4.1. Mechanical disruption

Mechanical disruption techniques, such as mechanical pressing, bead milling, and homogenization have all been proven and are utilized at large scales for cell disruption and other purposes (Greenwell et al., 2009; Mercer and Armenta, 2011). Mechanical pressing inflicts high pressures on the cells being extracted, forcing the cell wall to rupture, allowing the intracellular lipids to be extracted and collected. Pressing is a method commonly used for extraction of oil from plant seeds, but can be applied to microalgae (Mercer and Armenta, 2011). Homogenization achieves cell wall rupture by forcing the cells through a small orifice at high pressures. When the cell reaches the opening the sudden drop in pressure along with strong liquid shear forces causes the cell to break open allowing the lipids to be extracted (Greenwell et al., 2009). Bead milling, or bead beating, has been used at both laboratory and industrial scales for size reduction of particles and the disruption of cells (Doucha and Lívanský, 2008). This technique works by agitating algal biomass in the presence beads. Agitation allows the beads to pulverize the algal cells, breaking them apart by mechanical force and providing a means to extract the lipids (Mercer and Armenta, 2011).

In addition to mechanical pressing, homogenization, and bead beating methods of achieving algal cell disruption, algal biomass can be treated by autoclaving, exposure to microwaves, sonication, osmotic shock (Lee et al., 2010; U.S. DOE, 2010; Gong and Jiang,

2011). Such treatments can be used in conjunction with the mechanical disruption techniques to weaken algal cells and achieve higher lipid extraction efficiencies. Figure 2 illustrates the effectiveness of each of these methods compared to extracting lipids from non-disrupted algal cells. Use of microwaves was found to be the simplest and most effective for the extraction of algal oil by Lee et al. However, microwaves and sonication techniques may be difficult to scale up from laboratory scale to pilot or industrial scales (Mercer and Armenta, 2011).

4.2. Solvent extraction of lipids

Solvent extraction techniques are widely used and effective for the extraction of lipids from microalgae as well as vegetable seed, such as soybeans (Russin et al., 2010). This is due to the high solubility of lipids in non-polar solvents such as chloroform, hexane, petroleum ether, and others (Ahmad et al., 2011). A number of different standard extraction protocols exist such as the Folch extraction, Bligh and Dyer, and the Soxhlet or Gold-fisch techniques (Folch et al., 1957; Bligh and Dyer, 1959; Gloria et al., 1985). These methods provide standard solvents and ratios of solvents to use for the extraction of lipids from biomass or specific apparatuses for effective lipid extraction from various forms of biomass.

Although the use of solvents to extract algal lipids is fairly straightforward, there are a number of drawbacks when applied to microalgae. Standard methods such as the Folch and Bligh and Dyer methods of lipid extraction have proven inefficient when applied to microalgae. Oil extraction requires that water be removed from the biomass prior to lipid extraction for optimum results. If the biomass is not dried to a certain extent, water tends to interfere in the extraction process by shielding lipids from the extracting solvent (Converti et al., 2009; U.S. DOE, 2010; Young et al., 2010; Halim et al., 2011). Drying of algal biomass requires a large input of energy and can account of over 70% of the energy required to generate biodiesel from microalgae (Lardon et al., 2009).

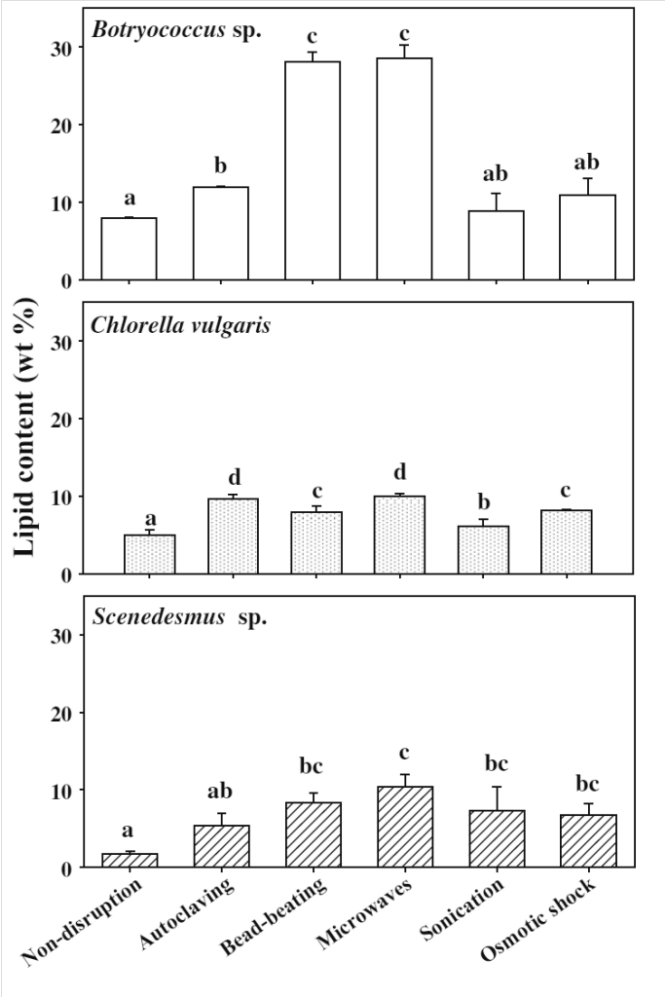

Figure 2. Evaluation of methods for microalgal lipid extraction (Lee et al., 2010).

Solvents are also biased in the class of lipids that dissolve; therefore, use of certain solvents does not effectively extract lipids from the algal biomass due to polarity mismatches (Guckert et al., 1988; Lewis et al., 2000; Mulbry et al., 2009; Samorì et al., 2010; Young et al., 2010). Hexane, a commonly used solvent for oil extraction, has been shown to extract a fraction of the lipids available in algae, depending on the lipids present in the biomass (Halim et al., 2011). Significant research efforts are being made to determine effective solvents, or combinations of solvents, capable of removing maximum amounts of lipids from algal biomass (Lewis et al., 2000; Dufreche et al., 2007; Converti et al., 2009; Mulbry et al., 2009; Russin et al., 2010; Young et al., 2010). Many times the solvents being used, or studied, are capable of

effectively extracting lipids from microalgae, but are too costly or difficult to used at large scales (Herrero et al., 2006; Campbell et al., 2010; Russin et al., 2010).

In many cases algal cells are resistant to solvent extraction, requiring physical or chemical pre-treatment in order to degrade the cells to effectively remove lipids (Molina Grima et al., 2003; Gouveia and Oliveira, 2008; Lee et al., 2010; Singh and Gu, 2010). Multiple studies have shown increases in lipid yield via solvent extraction after cell degradation (Converti et al., 2009; Widjaja et al., 2009; Lee et al., 2010). These methods, specifically microwave or ultrasonic assisted extraction or transesterification, are difficult to scale up and are energy intensive and will add to the biodiesel production costs when used (Pienkos and Darzins, 2009; Vyas et al., 2010).

Algal cells grown phototrophically, build up photosynthetic pigments, specifically chlorophylls and carotenoids (Nelson and Cox, 2005). These compounds are extracted along with the oil by solvents, resulting in contamination of the algal oil (Piorreck et al., 1984; Issariyakul and Dalai, 2010; Kanda and Li, 2011). Extracted pigments can be carried through the biodiesel producing process and require removal by purification processes to produce high quality biodiesel (U.S. DOE, 2010).

4.3. Supercritical fluid extraction

Supercritical fluid extraction of lipids has become an intensely studied area due to its ability to overcome many of the shortcomings associated with organic solvent based lipid extractions (Carrapiso and Garcia, 2000). Super critical fluid extraction typically makes use of carbon dioxide that has been pushed beyond its critical point to its supercritical state, as shown in Figure 3. At this phase the properties of carbon dioxide change, possessing the properties of both a liquid and a gas, making it more diffusive with low viscosity (Herrero et al., 2006; Gong and Jiang, 2011). Because of these changes the carbon dioxide is able to quickly penetrate solids and extract the target molecule(s) of interest. Other substances can be used as supercritical fluids, but

CO_2 is the most commonly used due to the relatively low critical temperature and pressure (31.1 ^oC and 72.9 atm) (Herrero et al., 2006; Mercer and Armenta, 2011).

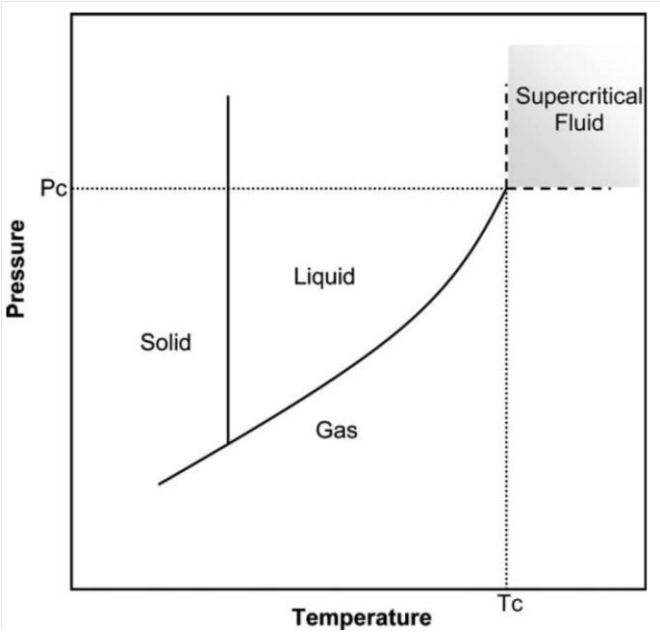

Figure 3. Phase diagram of a typical compound illustrating the conditions required to achieve the supercritical phase.

There are numerous advantages of using supercritical CO_2 for the extraction of algal lipids. The most commonly discussed is that organic solvents are not required (Carrapiso and Garcia, 2000). Solvent extraction of lipids requires large volumes of potentially toxic and hazardous compounds, whereas supercritical CO_2 extraction uses only carbon dioxide as the extracting solvent (Carrapiso and Garcia, 2000; Halim et al., 2011). Because CO_2 is a gas at ambient conditions, once the biomass has been extracted the solvent, CO_2, can be removed by evaporation leaving behind a pure extract with minimal to no contaminants (Mercer and Armenta, 2011).

In addition, the solvent strength of CO_2 can be tailored by altering the temperature and pressure of the extraction process, providing the means to extract compounds of various polarities or specific compounds (Herrero et al., 2006). Although the solvating power of CO_2 can be varied,

this technique struggles with extracting polar lipids. In order to overcome this problem, the addition of small quantities of co-solvents (10-15% ethanol) can improve the extraction yields (Mercer and Armenta, 2011). Solvents however, are typically fixed in their solvent power, based on their polarity.

A significant advantage of using supercritical CO_2 extraction is the ability to extract lipids from wet algal biomass. The use of organic solvents requires the dewatering of algal biomass to greater than 90% solids to avoid reductions in lipid yield (Lardon et al., 2009; U.S. DOE, 2010). However, supercritical CO_2 lipid extraction can achieve higher yields using wet algal biomass (30 wt% solids) than dried algal biomass according to Halim et al. (2011). The ability to extract lipids from wet algal biomass can save a significant amount of energy and this capability requires further research.

However, it is due to the high energy demand of supercritical fluid extraction processes that it is not a more widely used method for extracting lipids from microalgae (Carrapiso and Garcia, 2000; U.S. DOE, 2010; Halim et al., 2011). With advancements in this field, it may be possible to bring the energy requirements and equipment costs low enough to make this highly effective method of lipid extraction feasible on larger scales.

Although supercritical fluid extraction processes are highly efficient and fast, they remain expensive processes to build and require significant amounts of energy to operate (U.S. DOE, 2010; Halim et al., 2011). Mechanical methods such as pressing require large amounts of sample and are much slower (Singh and Gu, 2010). Therefore, solvent extraction and/or combined with mechanical disruption techniques remain the most commonly and widely used method to extract lipids from microalgal biomass due to their simplicity.

5. Biodiesel production

Once the lipids are extracted from the biomass, they must be converted to biodiesel. Biodiesel production from any lipid feedstock is performed via a chemical conversion process

known as transesterification (esterification for FFA). Oils are converted from their original form as triglycerides, FFA, or other complex lipids, to alkyl esters, which closely resemble petroleum based diesel, both chemically and physically (Ma and Hanna, 1999; Meher et al., 2006; Vyas et al., 2010). This conversion can be performed using a number of methods by reacting lipids with an alcohol with, or without, the presence of a catalyst, depending on the method (Ma and Hanna, 1999; Demirbas, 2006; Huang et al., 2010). Figure 4 illustrates the conversion of one mole of a glyceride molecule to 3 moles of fatty acid alkyl esters (FAMEs), or biodiesel, and one mole of glycerol in the presence of a catalyst.

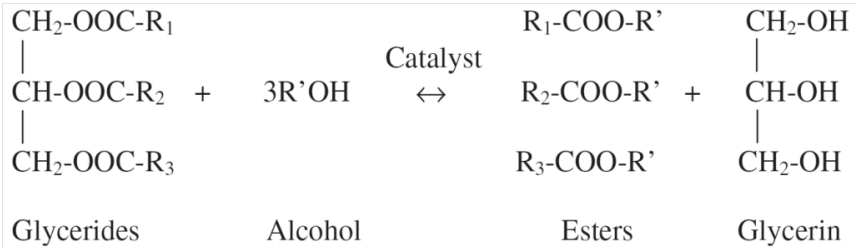

Figure 4. Conversion of lipids, or oil, to alkyl esters via transesterification (Marchetti et al., 2007).

Depending on the quality and type of lipid feedstock used, the concentration of free fatty acids can change (Canakci and Van Gerpen, 2001; Canakci and Van Gerpen, 2003; Meher et al., 2006; Canakci, 2007). Free fatty acids react differently than glyceride molecules, depending on whether an acid or a base is used as the catalyst, as shown in Figures 5 and 6 (Marchetti et al., 2007).

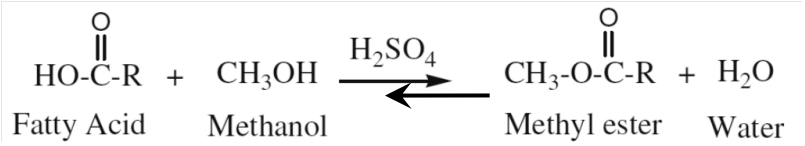

Figure 5. Free fatty acid reacting with methanol in the presence of a strong homogenous catalyst to form a methyl ester via esterification (Huang et al., 2010).

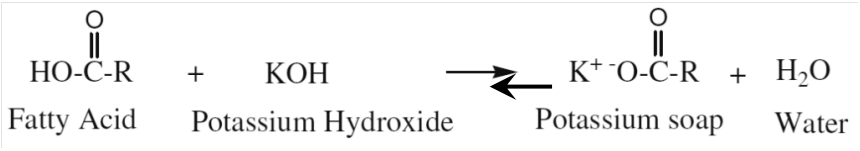

Figure 6. Free fatty acid reacting with potassium hydroxide to form the salt of the free fatty acid via the saponification reaction (Huang et al., 2010).

5.1. Acid Catalyzed Transesterification

Transesterification is generally performed using a homogeneous catalysts, which are catalytic compounds dissolved into the reaction medium. Homogenous acid catalysts used are strong acids such as hydrochloric, sulfuric, sulfonic, and phosphoric acids for example (Demirbas, 2009; Vyas et al., 2010). The strong acid is mixed into the alcohol, dissolved, and then contacted with oil allowing for the formation of the alkyl esters (Demirbas, 2009). A mechanistic illustration is provided in Figure 7 for acid catalyzed transesterification.

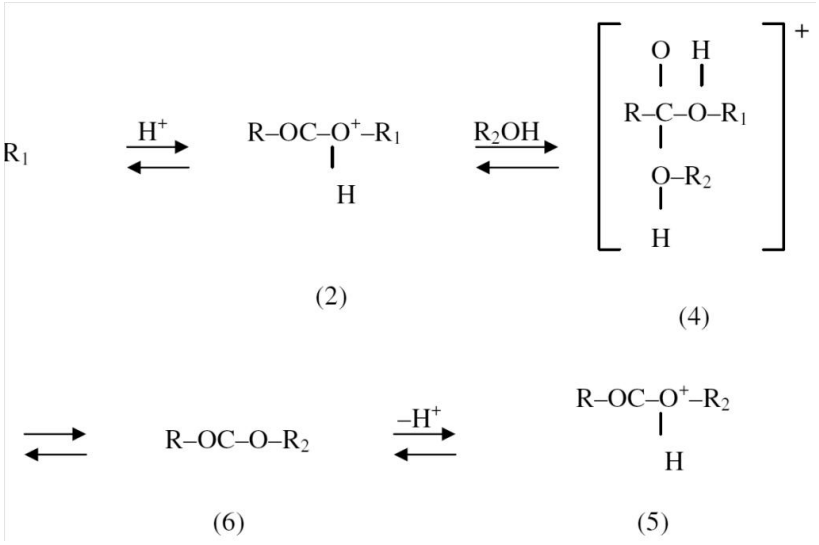

Figure 7: Mechanism of acid catalyzed transesterification of vegetable oils (Demirbas, 2009).

Specific parameters have been studied by a number of researchers including the type of alcohol and catalyst used, temperature, time, and reaction pressure (Freedman et al., 1986; Al-

Widyan and Al-Shyoukh, 2002; Meher et al., 2006; Azócar et al., 2010). In addition to these variables other relevant factors include the concentration of water as well as the amount of free fatty acids (FFAs) present in the oil (Ma et al., 1998; Kulkarni and Dalai, 2006; Canakci, 2007). Results from these studies and trends will be discussed.

Use of longer chain alcohols, such as butanol, has shown better results due to their ability to form a single phase with the oil. Shorter alcohols, such as methanol, are more polar and form two phases, which leads to slower reaction kinetics (Freedman et al., 1986; Meher et al., 2006). This can be explained by mass transfer limitations between the alcohol and oil phases when a two phase reaction is formed. If the reaction is a single phase the oil, alcohol, and catalyst are in constant contact facilitating the reaction (Ataya et al., 2007). However, due to the availability of methanol and ethanol and their lower cost, these alcohols are generally used over other alcohols (Lotero et al., 2005).

Focus has been placed more on hydrochloric and sulfuric acid as catalysts, and when compared, sulfuric acid has shown to provide higher levels of conversion and reaction rates (Al-Widyan and Al-Shyoukh, 2002; Meher et al., 2006). Based on these results, the use of methanol and sulfuric acid to carry out transesterification of algal oils is preferred and is the common means of generating biodiesel from the lipids (Al-Widyan and Al-Shyoukh, 2002; Kulkarni and Dalai, 2006; Miao and Wu, 2006; Johnson and Wen, 2009; Ehimen et al., 2010; Mata et al., 2010).

Methanol to oil ratio is an important factor as well as the catalyst concentration. With the FAME generating reaction being reversible, it is necessary to push the equilibrium to the right by adding excess methanol. Based on the stoichiometry, a ratio of 3: 1 molar ratio of methanol to oil should be used, as shown in Figure 4, but since the equilibrium needs to be shifted, the ratios used tend to be higher (30: 1) (Ma and Hanna, 1999). Table 5 illustrates various reaction conditions and resulting biodiesel yields. The trends indicate higher methanol to oil ratios and higher acid concentrations yield better lipid conversion (Meher et al., 2006).

The presence of water in the oil, or reaction medium, inhibits the reaction. Inhibition is widely explained by three mechanisms in the literature. Water tends to shield lipids away from the extracting solvent or methanol and catalyst in solution (U.S. DOE, 2010). This does not allow the alcohol and catalyst to contact the oil, therefore no reaction can occur (Lotero et al., 2005; Ataya et al., 2007). Secondly, biodiesel formation is a reversible reaction, as shown in Figure 5. In the presence of water, biodiesel can be hydrolyzed back to free fatty acids and the alcohol, and the equilibrium will be shifted to the reactant side as the concentration of water in the system increases (Liu et al., 2006). The final mechanism is the deactivation of the catalyst. When acids are used, the water takes up protons out of the solution. This is due to water being a better proton acceptor than the lipid molecules being targeted (Liu et al., 2006). Even 0.1 wt% water content in the oil can lead to a negative impact on the biodiesel yield. When the water content reaches 5 wt% of the oil, the reaction can become totally inhibited (Canakci and Van Gerpen, 2001). When FFAs are present in the lipid feedstock, water is generated via the reaction shown in Figure 5, thereby inhibiting FAME production. This trend and others presented in Table 5 are also observed in numerous other studies (Freedman et al., 1986; Boocock et al., 1996; Van Gerpen, 2005; Haas and Scott, 2006; Kulkarni and Dalai, 2006; Meher et al., 2006; Vyas et al., 2010).

5.2. Alkali Catalyzed Transesterification

It has been well documented that alkali catalyzed transesterification is faster than acid catalyzed transesterification, up to 4000 times faster (Vyas et al., 2010). Higher reaction rates are due to the strong nucleophilic nature of the alkoxides species formed from the catalyst and alcohol (Lotero et al., 2005). Several catalysts can be used including sodium hydroxide, potassium hydroxide, and sodium methoxide (Meher et al., 2006). A mechanistic illustration of the base catalyzed reaction is provided in Figure 8. Quicker reaction rates allow for economically feasible production of biodiesel from vegetable oils on commercial scales with yields of close to 100% of the maximum (Ma and Hanna, 1999; Vyas et al., 2010). Varying the reaction

Table 5. Effect of varying parameters on the conversion of oil to Methyl Esters using an acid catalyst. Adapted from Kulkarni et al. (Kulkarni and Dalai, 2006).

Parameter	Molar Ratio (methanol: Oil)	Catalyst (H_2SO_4) (%)	FFA (%)	Temperature (^oC)	Time (hrs)	Water (%)	Conversion (%)
Effect of Temperature:	6 to 1	3	0	25	48	0	~10
				45			~55
				60			~85
Effect of Reaction Time:	6 to 1	3	0	60	48	0	~88
					96		~95
Effect of Molar Ratio:	3.3 to 1	3	0	60	48	0	~77
	3.9 to 1						~80
	6 to 1						~87
	20 to 1						~95
	30 to 1						~98
Effect of Catalyst Concentration:	6 to 1	1	0	60	48	0	~72
		3					~88
		5					~95
Effect of FFA:	6 to 1	3	5	60	96	0	~90
			10				~88
			15				~80
			20				~73
			33				~60
Effect of Water:	6 to 1	3	0	60	96	0.1	~92
						0.5	~90
						1	~82
						3	~32
						5	~5

parameters, such as temperature, alcohol, and catalyst concentration results in similar trends to when acids catalysts are used (Freedman et al., 1986; Van Gerpen, 2005; Meher et al., 2006; Demirbas, 2009; Azócar et al., 2010; Vyas et al., 2010).

However, base catalysts have a number of drawbacks that make their use complicated based on the lipid feedstock. One of the major drawbacks is the presence of water and/or FFAs in the feedstock (Meher et al., 2006). Water again severely inhibits the reaction, by reducing the effectiveness of the catalyst due to salt formation (Meher et al., 2006).

Presence of water leads to the hydrolysis of the oil and subsequent neutralization of the resulting free fatty acids (FFAs) to soap. If FFAs are present in the oil, they are immediately converted to soaps, leading to a loss of catalyst, as well as to difficulties in downstream purification of the biodiesel. The levels of water and FFAs tolerable in the oil should be between 0.1 – 0.3 wt% and less than 0.5 wt% respectively (Lotero et al., 2005). The effect that FFA has on base catalyzed transesterification is illustrated in Figure 9. These tight tolerances require low quality oils to be refined, or the oil feedstock to be highly pure, which leads to added costs for the production of biodiesel.

For application to algal oils, base catalysts are typically not used due to the high levels of FFA in microalgal oils. Studies have shown that algal lipids contain some amount of FFA, which could lead to a loss of conversion efficiency (Meher et al., 2006; Miao and Wu, 2006). With certain microalgae, the levels of FFA is lower, about 0.6% by dry mass (Samorì et al., 2010), possibly allowing for the use of base catalysts. In cases where FFA content is high, acid catalysts are preferred with sulfuric acid being the most commonly used, based on its performance(Miao and Wu, 2006; Johnson and Wen, 2009; Vicente et al., 2009; Wahlen et al., 2011).

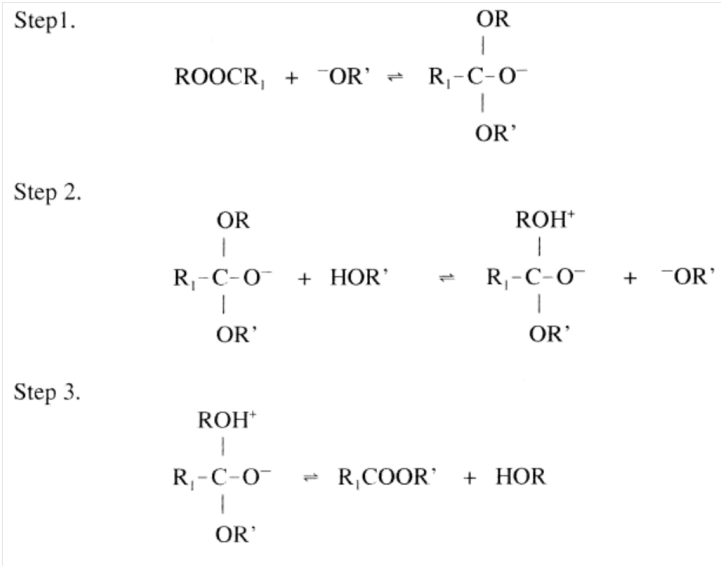

Figure 8: Base catalyzed transesterification (Ma and Hanna, 1999).

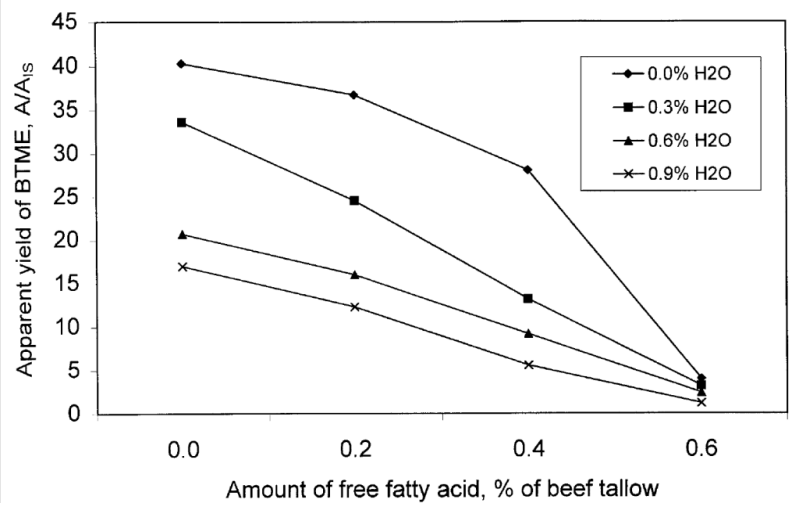

Figure 9: Effect of FFA on base catalyzed transesterification of beef tallow (Ma et al., 1998).

5.3. Enzymatic Transesterification

Lipases are enzymes that have the capacity to hydrolyze complex lipids, such as triacylglycerols, as well as catalyzing the conversion of these lipids to alkyl esters, or biodiesel, in the presence of an alcohol. They possess a number of advantages over standard acid or base catalyzed transesterification methods (Fukuda et al., 2001; Meher et al., 2006; Marchetti et al., 2007; Demirbas, 2009; Vyas et al., 2010).

Because some oil feedstocks contain water, and further purification of the oil adds to the production cost of biodiesel, it is advantageous to use oil with moisture. Enzyme catalysts allow for this because of their need for water, without which, the enzymes remain inactive (Vyas et al., 2010). When homogeneous catalysts are used, many times the catalyst is lost when neutralized or discarded downstream of the reaction (Marchetti et al., 2007; Demirbas, 2009). Enzyme catalyst can be immobilized on a substrate allowing for repeated utilization. This not only saves cost, but the immobilization has shown enhanced conversion of lipids over non immobilized enzymes (Azócar et al., 2010). Transesterification of lipids via enzyme catalysts leads to lower concentrations of contaminants in the crude biodiesel, due to enzyme specificity. Therefore, the

risk of side reactions and unwanted byproducts is reduced, resulting in less effort for downstream purification of the crude biodiesel (Fukuda et al., 2001; Marchetti et al., 2007; Vyas et al., 2010).

Use of lipases allow for the reduction in reaction temperature by reducing the energy requirements of the reaction (Fukuda et al., 2001). Finally, lipases are unaffected by the presence of water and FFA in the oil and are able to esterify FFAs to alkyl esters. Conversion rates greater than 95% are achievable with enzyme catalyzed transesterification (Kulkarni and Dalai, 2006).

Although the advantages to using enzymatic catalysts are numerous, large scale use of lipases for biodiesel production has not been practiced (Demirbas, 2009). Production of enzymes is costly and can drive up biodiesel production costs (Meher et al., 2006; Demirbas, 2009). Until the production costs of enzyme catalysts are reduced and the process streamlined, this method will continue to remain too costly to scale up (Vyas et al., 2010).

5.4. Supercritical Transesterification

Use of supercritical solvents has been considered a potential method for the simultaneous extraction and conversion of oils from biomass to biodiesel and for the extraction of high value pigments and compounds (Herrero et al., 2006; Marchetti et al., 2007; Sharma et al., 2008; Singh and Gu, 2010). This method of extraction and conversion makes use of solvents, such as methanol or ethanol, which are beyond their critical point, as shown in Figure 3. When a compound reaches this phase, its physical properties change allowing them to penetrate solids and effectively dissolve compounds not soluble in the solvent at normal conditions. Changes in the solvent's properties allow for super critical solvents to break down cell matter, dissolve oils or other desired products, and extract the target compounds much more efficiently and quickly (Demirbas, 2006; Herrero et al., 2006; Levine et al., 2010; Halim et al., 2011).

Supercritical transesterification can be performed without a catalyst due to the catalytic nature of the alcohol at the supercritical state (Vyas et al., 2010). At this state the dielectric constant of methanol, for example, decreases, lowering its polarity and allowing it to become

soluble in the oil phase (Sharma et al., 2008; Vyas et al., 2010). The alcohol and the oil form a single reaction phase enabling faster reaction rates (Vyas et al., 2010). At conditions below the critical point of methanol, methanol is not soluble in the oil, resulting in a two phase reaction system (Fukuda et al., 2001). With this technique, conversion levels of 95% in approximately 10 minutes can be achieved, at specific reaction conditions (Sharma et al., 2008).

The extractive ability of supercritical solvents and the high conversion rates of the oil to biodiesel make this approach attractive. Biodiesel generated is extremely pure requiring little purification after the reaction (Vyas et al., 2010). Pushing the reaction to reach supercritical states however, requires significant amounts of energy and an apparatus able to withstand the high temperatures and pressures required (Fukuda et al., 2001; Ehimen et al., 2010).

Table 6. Comparison of transesterification methods. (SCM – Supercritical Methanol) (Demirbas, 2006).

	Catalytic MeOH Process	SCM Method
Methylating Agent	Methanol	Methanol
Catalyst	Alkali	None
Reaction Temperature (K)	303-338	523-573
Reaction Pressure (Mpa)	0.1	10-25
Reaction Time (min)	60-360	7-15
Methyl Ester Yield (wt%)	96	98
Removal for purification	Methanol, catalyst, glycerol, soaps	Methanol
Free fatty acids	Saponified products	Methyl esters, water
Continuity easiness	Discontinue	Easy continuity

Ethanol, for example, requires temperatures of 573K and 20 MPa when used as a supercritical solvent (Sharma et al., 2008). Research has focused on reducing these harsh

conditions to develop a process that consumes less energy (Sharma et al., 2008). This has involved the addition of co-solvents and small quantities of catalyst (Vyas et al., 2010). Table 6

Table 7. Comparison of transesterification methods (Marchetti et al., 2007).

Variable	Alkali catalysis	Lipase catalysis	Supercritical alcohol	Acid catalysis
Temperature (^{o}C)	60-70	30-40	239-385	55-80
Free fatty acid in raw material	Saponified products	Methyl Esters	Esters	Esters
Water in raw material	Interference with reaction	No influence	-	Interference with reaction
Yield of methyl esters	Normal	Higher	Good	Normal
Recovery of glycerol	Diffiuclt	Higher	-	Normal
Purification of methyl esters	Repeated washings	None	-	Repeated washing
Production cost of catalyst	Cheap	Relatively expensive	Medium	Cheap

presents a comparison of supercritical transesterification of vegetable oils versus base catalyzed transesterification, while Table 7 presents a comparison of all transesterification methods discussed thus far. With current methods and technologies the supercritical transesterification process is not yet scalable, but shows promise if the energy and cost requirements can be reduced.

5.5. In situ Transesterification

In situ transesterification was originally intended as a method to accurately and quickly quantify the total lipid content of biomass of interest and has been used for multiple forms of biomass (Indarti et al., 2005; Lepage and Roy, 1986; Griffiths et al., 2010). It has become an intensely studied method because of its ability to simultaneously extract and convert lipids from whole cell biomass (Liu and Zhao, 2007; Ehimen et al., 2010; Griffiths et al., 2010; D'Oca et al., 2011; Wahlen et al., 2011). *In situ* transesterification makes use of the same basic principles to

convert lipids to biodiesel, outlined in previous sections, but does not require the oil to be previously extracted from the biomass. Without the need for a separate solvent extracting step, processing algal biomass becomes simpler, while maintaining high biodiesel yields (Xu and Mi, 2010).

In situ transesterification works by contacting dried algal biomass with an alcohol and strong catalyst dissolved within it (Carrapiso and Garcia, 2000; Nelson, 2010). This combination works to degrade the algal cells and bring lipids from within the cell into solution. As this occurs, the complex lipids such as triglycerides, phospholipids, and other complex lipids are split by alcoholysis generating alkyl esters (Carrapiso and Garcia, 2000; Griffiths et al., 2010; Kargbo, 2010).

Alkyl esters generated are extracted from the reaction medium by liquid-liquid extraction using an organic solvent, one that is not miscible with the alcohol used in the reaction. The organic solvent is added to the reaction suspension and allowed to draw the hydrophobic FAMEs into the solvent phase (Dufreche et al., 2007; D'Oca et al., 2011; Wahlen et al., 2011). The solvent phase can be collected and analyzed for biodiesel content or purified to obtain usable biodiesel.

Multiple studies have shown the effectiveness of *in situ* transesterification of algal biomass over traditional biodiesel production methods via solvent extraction followed by transesterification of the extracted oil (Figure 10) (Indarti et al., 2005; Griffiths et al., 2010; Wahlen et al., 2011). This efficiency lies in the combination of the catalyst with the alcohol contacting the algal biomass, which is evidenced by studies conducted by Whalen et al, presented in Table 8 (Wahlen et al., 2011). Several variations of this method as well as conditions have also been studied, many of which have led to increased biodiesel yields from microalgae (Carrapiso and Garcia, 2000; Haas and Scott, 2006; Johnson and Wen, 2009; Vicente et al., 2009; Ehimen et al., 2010).

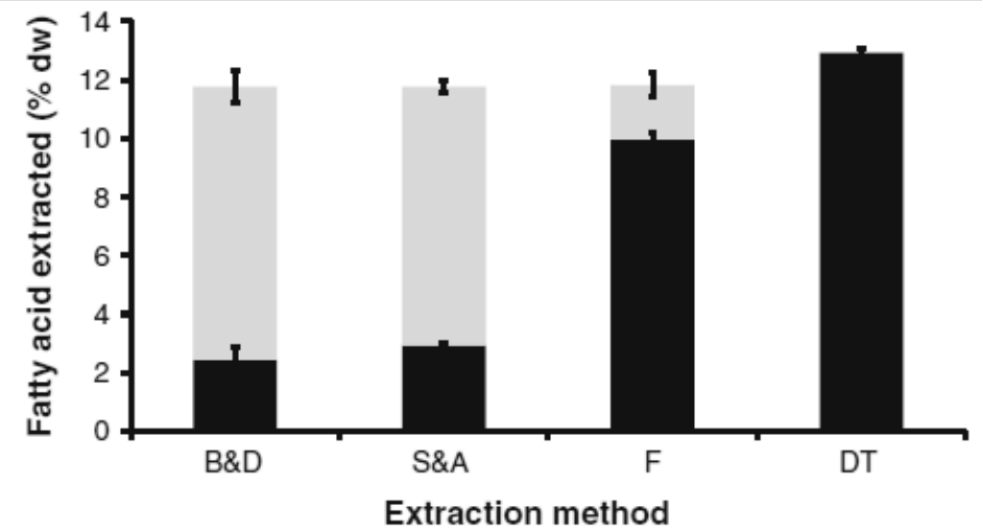

Figure 10. Comparison of *in situ* or direct transesterification with traditional oil extracting methods for *Chlorella vulgaris* (Griffiths et al., 2010). B&D refers to Bligh and Dyer, S&A refers to Smedes and Askland, F refers to Folch, and DT refers to direct transesterification. Black bars represent lipids collected by the method indicated. Gray bars represent remaining biomass post extraction.

Table 8. Comparison of lipids extracted by solvents versus FAMEs generated by *in situ* transesterification. TAG refers to triacylglycerol. Adapted from Whalen et al. (2011).

Alcohol	mg TAG extracted[a]	mg FAME per sample[b]
Methanol	3.1	35.6
Ethanol	20.2	30.8
1-butanol	18.9	36.9
2-methyl-1-proponal	19.5	28.7
3-methyl-1-butanol	19.1	36.4

[a] 100 mg biomass extracted with 1 mL of alcohol heated to 60^{o}C by microwave irradiation for 10 min with constant stirring.
[b] 100 mg of biomass was heated to 60^{o}C for 100 min with 2 mL of alcohol and 1.8% (v/v) sulfuric acid.

A major disadvantages associated with this method is the need to use dried algal biomass. Drying of the algal biomass can be achieved using a number of different methods, however many require large amounts of energy. Dewatering and drying of algal biomass is energy intensive and costly, but the presence of moisture severely inhibits the production of biodiesel (Liu et al., 2006;

Carrapiso and Garcia, 2000; Haas and Scott, 2006; Johnson and Wen, 2009; Ehimen et al., 2010; Griffiths et al., 2010).

Even though there is no separate oil extraction step, pigments continue to be extracted into the organic solvent phase that also contains FAMEs (U.S. DOE, 2010). This necessitates further purification of the generated crude biodiesel. Although the presence of moisture is a hindrance, *in situ* transesterification currently shows the most promise of becoming a scale-able process for the production of biodiesel from microalgae. Cost savings are possible due to the removal of the oil extracting step, which simplifies the process and reduces the amount of material required to generate biodiesel from microalgae (Johnson and Wen, 2009; Nelson, 2010; Xu and Mi, 2010).

6. Conclusions

Each of the major phases of algal biodiesel production has been covered in this literature review with each having an impact on the feasibility of producing algal biodiesel economically on a large scale. The first phase of the process is growth of the algal biomass. There are two classic options available for large scale growth of algal biomass, these being open and closed systems (Harun et al., 2010). Open systems have been widely recognized as the most feasible approach to growth of large quantities of biomass because of its low capital investment, low maintenance requirements, and overall ease of operation (Harun et al., 2010). For economically viable biodiesel production, open systems should be used, until closed systems are optimized enough for large scale production of algae at reasonable cost.

Collection of the algae grown can be performed using various methods including centrifugation, filtration, or sedimentation. Each method has associated costs and drawbacks with centrifugation being too energy intensive (Lardon et al., 2009). Filtration methods suffer from operational costs and lack the speed required at large scales (Molina Grima et al., 2003). Sedimentation methods require the use of some form of flocculent, which may be costly

depending on the amount added and the type of flocculent used. In addition, removal of flocculent in the spent water can become complicated (Molina Grima et al., 2003). Combinations of these methods may be the most effective option to reduce the energy usage and cost of harvesting algal biomass.

Once the algal biomass has been grown and collected it can be processed to biodiesel through a number of different methods. These include oil extraction-transesterification or *in situ* transesterification processes (Mata et al., 2010). The efficiencies of both methods depend on the amount of moisture remaining in the biomass after harvesting (Ehimen et al., 2010). Options exist for the processing of algae that contains moisture; however, they typically require the use of super or sub critical liquids (Levine et al., 2010; Halim et al., 2011). These processes are energy intensive, costly to build, and therefore difficult to scale up. Solvent extraction followed by transesterification requires large volumes of costly solvents that are in many cases toxic (Nelson, 2010). In addition, many solvent extraction protocols are not effective in removing lipids from the algal biomass. Of these methods, the *in situ* transesterification procedure provides the most cost effective method (Johnson and Wen, 2009). It allows for simultaneous extraction and transesterification of algal lipids. This negates the need for a separate solvent extraction step and reduces the amount of energy and materials needed for biodiesel production (Ehimen et al., 2010).

From this review major hurdles that have been identified involve the inhibition that water creates in the conversion of lipids to biodiesel. Thermal dewatering of algae is costly and adds to the cost of biodiesel production (Lardon et al., 2009). Additional hurdles involve the presence of chlorophyll and other photosynthetic pigments in the biodiesel generated from algae grown under phototrophic conditions (U.S. DOE, 2010). Chlorophyll contamination requires further purification of the oil and/or biodiesel, which may be avoided if the chlorophyll contamination can be reduced or removed. Whichever process is used for the production of biodiesel, the overall production cost needs to be lowered to make it competitive with petroleum based diesel fuels.

Without a reduction in the cost of harvesting algal biomass from dilute suspensions, processing the algal biomass for lipid extraction, conversion of the extracted lipids, and production of high quality biodiesel producing algal based biodiesel will be limited. This is evident due to the lack of commercial production facilities for algal biodiesel (Lardon et al., 2009). As these hurdles are solved algal biodiesel will come closer to becoming a mainstream liquid fuel.

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CHAPTER 3

BIODIESEL PRODUCTION VIA A WET LIPID EXTRACTION PROCEDURE[1]

1. Introduction

Dependence on petroleum based fuels is not sustainable due to increasing fuel costs, steady depletion of crude oil, and the environmental consequences associated with the use of fossil fuels (Chisti, 2007; Demirbas and Fatih Demirbas, 2011; Schenk et al., 2008). One option for the production of renewable liquid fuels is biodiesel from microalgae to offset usage of crude oil based diesel (Demirbas and Fatih Demirbas, 2011). Microalgae possess advantageous characteristics that warrant its consideration as a source of alternative oil for biodiesel production, as well as a feedstock for the production of additional biofuels and bioproducts (Christenson and Sims, 2011; Mata et al., 2010).

Processes exist for the extraction and/or conversion of algal oils to biodiesel including organic solvent extraction, super-critical fluid extraction, and direct transesterification (Ehimen et al., 2010; Gong and Jiang, 2011). Solvent based lipid extraction and direct transesterification techniques are inhibited when performed in the presence of a water phase (Ehimen et al., 2010; Griffiths et al., 2010). However, dewatering and drying algae is both costly and energy intensive (Molina Grima et al., 2003; Lardon et al., 2009). Traditional solvent based lipid extraction procedures also extract pigments such as chlorophyll. Chlorophyll and the associated magnesium are contaminants of algal lipid extracts and can reduce the quality of the produced biodiesel (de Jesus et al., 2010; Moser, 2009; U.S. DOE, 2010). Super critical fluid methods are able to process wet algal biomass for oil extraction and/or transesterification at high efficiencies, but are currently considered too costly to scale up and operate (Halim et al., 2011).

[1] Additional Coauthors: Ronald C. Sims

The aim of this study was to develop a method of lipid extraction that does not require drying of the harvested algal biomass and that also removes chlorophyll contamination prior to collection of algal lipids for conversion to biodiesel, or fatty acid methyl esters (FAMEs).

2. Materials and Methods

2.1. Chemicals and reagents

Reagents used in this study include ACS grade sulfuric acid from EMD Chemicals (Gibbstown, NJ), sodium hydroxide from Avantor Performance Chemicals (Center Valley, PA), and methanol obtained from Pharmco-AAPER (Brookfield, CT). HPLC grade hexanes was obtained from Fisher Chemicals (Pittsburgh, PA). Fatty acid methyl ester (FAME) standards were obtained from Supelco Analytical (Bellefonte, PA). All macronutrients used for media were laboratory or ACS grade, while micronutrients were technical or laboratory grade.

2.2. Algae growth and collection

Microalgae used for this study were obtained from the City of Logan, Utah municipal lagoon wastewater treatment facility, and grown indoors in well mixed 15L bioreactors as mixed cultures. *Chlorella* and *Scenedesmus* sp. accounted for the majority of the species (Griffiths, 2009). Lighting was supplied via GE Plant and Aquarium Ecolux lights with total light intensity of 1250 μmol m^2 s^{-1} for a period of 14 hours per day. A slightly modified version of the SE media (Li et al., 2008) was used as the growth media.

Algal biomass was harvested from the media by centrifugation and thoroughly mixed to account for potential variability in algal lipid content between the three reactors. From the mixed biomass, five samples were removed and lyophilized to determine the average moisture content of the harvested algal biomass, based on the mass of water removed. The lyophilized algal biomass was then properly stored for later testing. The remaining centrifuged algal biomass was immediately preserved as centrifuged at -80°C.

2.3. Wet lipid extraction procedure (WLEP)

The centrifuged algal biomass was found to contain 83.88 ± 0.75 wt % moisture. This biomass was used for evaluating the wet lipid extraction procedure (WLEP), illustrated in Figure 11.Six replicates of 100 mg dry mass equivalent samples of wet algal biomass were used to evaluate the procedure, as described in the following sections.

2.3.1. Acid and base hydrolysis of algal biomass

Acid hydrolysis was accomplished by adding wet algal biomass (100 mg dry mass equivalent) to separate glass tubes with 1 mL of a 1 M sulfuric acid solution. The tubes were sealed using PTFE lined screw caps, mixed, and heated to 90°C using a Hach DRB-200 heat block for 30 minutes with mixing provided at 15 minutes. These conditions allowed for the disruption of the algal cells in order to hydrolyze complex algal lipids to free fatty acids.

Following acid hydrolysis, 1.0 mL of a 5 M sodium hydroxide solution was added to each sample. The samples were subjected to heating at 90°C for 30 minutes. The addition of sodium hydroxide neutralized and transformed free fatty acids to their salt forms and saponified any remaining complex lipids. The samples were cooled and centrifuged to pellet the residual algal biomass. The lipids remained in their salt form dissolved in the aqueous phase by maintaining a high pH during centrifugation, thereby isolating them from association with the digested algal biomass.

The resulting supernatant phases were removed and collected from each sample in separate tubes, and the residual hydrolyzed biomass pellet was vigorously mixed with 1 mL deionized water. The resulting suspension was re-centrifuged and the liquid phase again removed and added to the corresponding tubes containing the original supernatant. The residual hydrolyzed algal biomass was removed as a side stream shown in Figure 11 (stream 1).

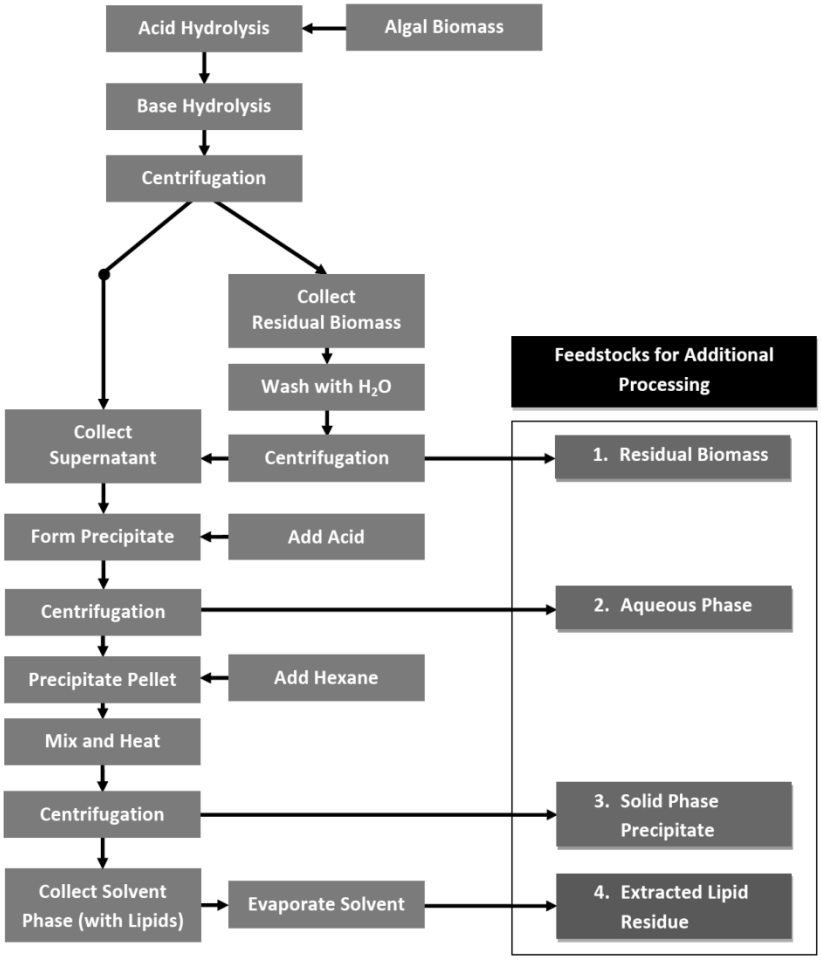

Figure 11. Block diagram depicting the wet lipid extraction procedure.

2.3.2. Chlorophyll precipitation followed by lipid extraction from the precipitated solids

To the supernatant phases collected in the previous step, 3.0 mL of a 0.5 M sulfuric acid solution was added to form a solid precipitate. The addition of the acidic solution lowered the pH below seven, allowing for the salts of the free fatty acids to revert back to their free fatty acid form. In addition, the decrease in pH produced a solid precipitate. Due to the free fatty acids' insolubility in an aqueous solution, the lipids associated with the precipitating solids.

The resulting solid-liquid suspension was centrifuged, the supernatant removed as the aqueous phase (stream 2) in Figure 11, and the precipitated solids were collected. To the tubes containing the collected solid precipitate, 5 mL of hexanes was added. The samples were sealed,

mixed, and heated to 90°C for 15 minutes with mixing provided every 5 minutes. Heating the collected precipitate in the presence of hexane allowed for the partitioning, or separation, of lipids from the solid phase into the solvent phase, while the chlorophyll remained within the solid phase.

After heating, the samples were cooled and centrifuged. The hexane phases were collected and transferred to separate tubes. Gentle heating was applied to the hexane phase under a filtered air stream to vaporize the solvent, leaving behind the extracted lipid residue, labeled "Extracted Lipid Residue" in Figure 11 (stream 4). The precipitated solids were removed from the procedure as the "solid phase precipitate" (stream 3).

2.4. Quantification of lipids in streams 1 through 4 from the wet lipid extraction procedure

A mass balance for lipids was determined by accounting for all transesterifiable lipids present in streams 1 – 4. Therefore, streams 1 through 4 were collected and analyzed for lipid content. Quantification of transesterifiable lipids was achieved by esterification of the lipids associated with each stream to methyl esters for subsequent analysis by gas chromatography.

Streams 1 through 3 were lyophilized prior to esterification to remove residual water within the samples in order to achieve complete conversion of lipids to methyl esters for an accurate mass balance analysis. Stream 4 is solvent based and did not need to be lyophilized. Lipids in each stream were esterified to FAMEs by the addition of 1 mL of a 5% (v/v) sulfuric acid solution in methanol directly to the corresponding test tubes containing streams 1, 2, 3 or 4.

For the reaction step, the test tubes were sealed, mixed, and heated at 90°C for 30 minutes to complete the conversion of the lipids within the sample to FAMEs. FAMEs were extracted from the acidified methanol phase after the reaction by adding 5 mL of hexanes and heating at 90°C for an additional 15 minutes. The resulting hexane phase containing FAMEs was collected for analysis by gas chromatography (GC).

2.5. Direct transesterification of algal biomass

Direct transesterification is a procedure commonly cited and used in the literature to convert lipids within algal biomass to methyl esters for quantification, and is considered a candidate procedure for large scale biodiesel production from microalgae (Ehimen et al., 2010; Griffiths et al., 2010). Previously lyophilized algal biomass, from section 2.2, was directly transesterified. Results obtained from the direct transesterification of lyophilized algal biomass served to both quantify the lipids present in the algal biomass used in this study, and to serve as a positive control for comparison to the wet lipid extraction procedure. Six 100 mg samples of lyophilized algal biomass were directly transesterified using 1 mL of a 5% (v/v) solution of sulfuric acid in methanol. The reaction and FAME extraction steps were conducted as described in section 2.4.

2.6. Gas Chromatography

Collected hexane phases, containing FAMEs, were analyzed using an Agilent 7890A GC equipped with an FID detector. A Restek Stabilwax-DA column (Bellefonte, PA) (30m x 0.32 mm id x 0.25μm film thickness) was used to separate individual FAME compounds. Helium was used as the carrier gas at a constant flow rate of 2 mL/min. The oven was held at 100°C for 1 minute then ramped to 235°C at a rate of 10°C /min and held for 10 minutes. The front inlet was operated in splitless mode, with an initial temperature of 100°C for 0.1 minutes and then increased to 235°C at a rate of 720°C /min and held for 5 minutes. Injection volume was set at 1 μL. FID temperature was maintained at 240°C.

Concentrations of individual FAMEs were determined by comparing sample peak areas with linear concentration correlations generated by the serial dilution of a C-8 to C-24 FAME mixture from Supelco Analytical (Bellefonte, PA). The total mass of FAMEs generated was based on the volume of hexane used to extract the FAMEs, after the esterification or transesterification reaction, and the concentration of FAMEs measured in the hexane phase.

2.7. Spectrophotometric analysis

Chlorophyll analysis was accomplished with a Shimadzu UV-1800 spectrophotometer from 300 to 900 nm. To confirm the precipitation of chlorophyll in stream 3, from Figure 11, the solid precipitate was collected, lyophilized, and dissolved in 5 M sodium hydroxide for analysis. A 5M sodium hydroxide solution was used as a blank for comparison. Freeze drying of the solid precipitate was performed specifically for tests involving chlorophyll detection and is not part of the protocol described in section 2.3.

Crude biodiesel phases produced from wet algal biomass, using both the direct transesterification and the wet lipid extraction procedure (WLEP), were also similarly analyzed for the presence of chlorophyll. Crude biodiesel refers to the hexane phase containing FAMEs extracted from the methanol reaction phase as described in section 2.4. Processing of wet algal biomass performed in this section is not intended for lipid quantification, but rather to illustrate contamination of the crude biodiesel phases by chlorophyll.

For the WLEP, crude biodiesel was obtained by converting lipids separated from the lyophilized precipitated solids to methyl esters. Direct transesterification of wet biomass (100 mg dry mass equivalent) provided crude biodiesel from the direct transesterification method for comparison. Crude biodiesel from the direct transesterification method required a 1 to 10 dilution due to the higher level of chlorophyll contamination, whereas the crude biodiesel generated from lipids extracted from the precipitated solids (stream 3) using the WLEP required no dilution.

3. Results and Discussion

Capabilities of the WLEP to precipitate chlorophyll and to extract lipids from wet algal biomass were evaluated and are presented in the following sections.

3.1. Removal of chlorophyll

To confirm that the chlorophyll was partitioned away from the liquid phase containing the lipids and remained associated with the solid precipitate phase (section 2.3.2), the precipitate

was re-dissolved in 5 M sodium hydroxide (section 2.7) and the optical density of the solution was measured. Absorbance peaks were found to occur at wavelengths characteristic of chlorophyll (Nelson and Cox, 2005), as shown in Figure 12.

To further illustrate chlorophyll removal, or reduction, crude biodiesel generated from wet algae using both the direct transesterification and WLEP were analyzed using a spectrophotometer. Figure 13 illustrates the difference in the absorbance properties between the crude biodiesel phases obtained using the two methods. These results show that the extraction of FAMEs generated using the direct transesterification of wet algal biomass extracted chlorophyll, along with the FAMEs, into the hexane phase. However, the WLEP accomplished the removal of chlorophyll through prior precipitation, thereby eliminating or reducing chlorophyll contamination of the crude biodiesel.

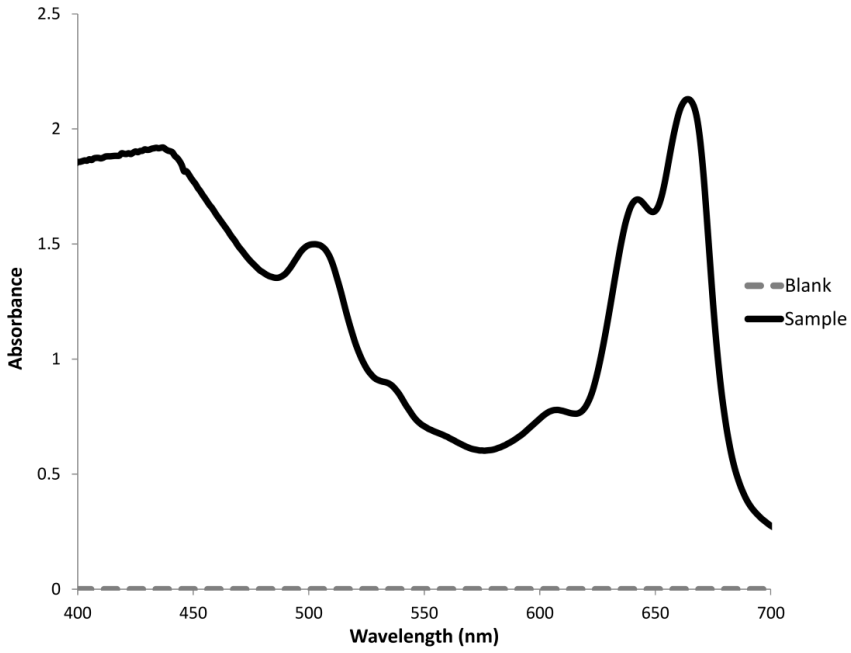

Figure 12. Absorbance spectrum for re-dissolved precipitate indicating the accumulation of chlorophyll in the solid precipitate. Absorbance peaks shown above are similar to photosynthetic chlorophyll (Nelson and Cox, 2005).

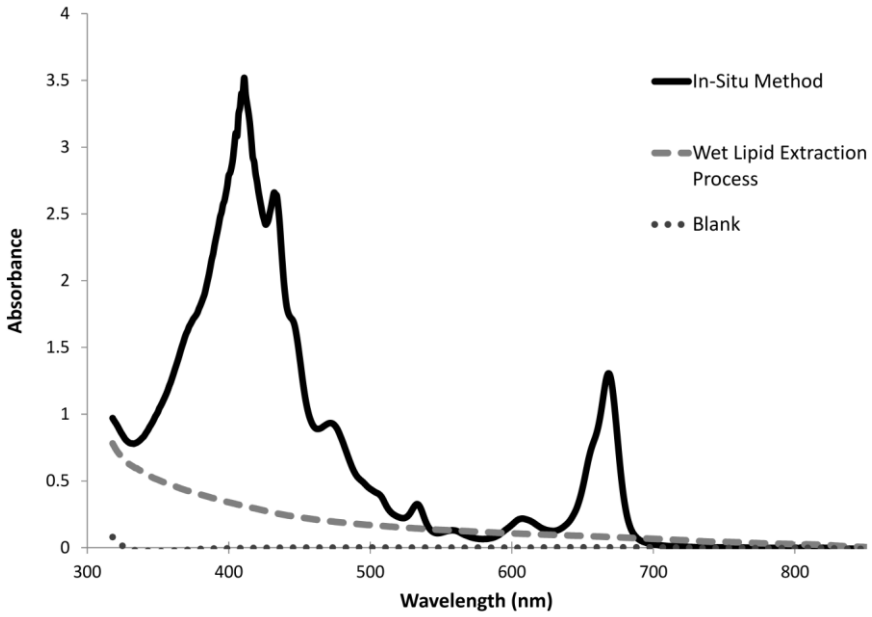

Figure 13. Comparison of the absorbance properties for crude biodiesel phases generated using the water based lipid extraction and direct transesterification methods for wet algal biomass.

3.2. Evaluation of the efficiency of the WLEP

To evaluate the efficiency of the WLEP to extract lipids, a mass balance on lipids was determined by measuring the mass of FAMEs generated from each side stream for each of the six replicates. Additionally, lyophilized algal biomass was transesterified using the direct transesterification method (section 2.5), and the FAME yield provided the maximum mass of FAMEs producible from the algal biomass used in this study and served as the positive control value.

Results of the mass balance for FAMEs are presented in Table 9. The mass of FAMEs recovered from streams 1 – 4 totaled 10.35 ± 0.35 mg, compared to the positive control value of 11.12 ± 0.26 mg FAME. These results show that essentially all transesterifiable lipids are accounted for in streams 1 – 4 of the WLEP.

Table 9. FAMEs generated from streams 1 through 4 from the WLEP (Figure 11) and from the positive control. All reported FAME masses are the average of six replicates.

FAMEs Produced:	mg FAME:	% of Control:
Direct Transesterification: (positive control)	11.12 ± 0.26	100%
Wet Extraction (total):	10.90 ±0.35	98.0%
From residual biomass: (1)	*2.29 ±0.08*	*20.6%*
From water phase: (2)	*0.13 ±0.00*	*1.1%*
From precipitate: (3)	*1.89 ±0.59*	*17.0%*
From hexane phase: (4)	*6.60 ±0.85*	*59.3%*

The residual biomass removed from the WLEP as stream 1 generated 2.29 mg of FAME, corresponding to 20.6% of the positive control value. Therefore, the remaining lipids (79.4%) are outside of the biomass, or extracted from the biomass. This result indicates that 79.4% of the transesterifiable lipids were extracted, or removed, from the wet algal biomass during the acid and base hydrolysis steps of the WLEP (section 2.3.1).

Streams 2, 3, and 4 were also evaluated for FAME production as part of the mass balance analysis described above. Streams 2 and 3 produced 2.02 mg of FAME or 18.1% of FAMEs produced by the positive control. Finally, stream 4, was found to contain enough transesterifiable lipids to generate 6.60 mg of FAME, corresponding to 59% of the positive control value. With the objective of the WLEP to extract lipids from the wet algal biomass and isolate those lipids in stream 4, the WLEP was found to be approximately 60% efficient.

3.3. Potential reduction in organic solvent required for algal lipid collection

Hexane is required in order to separate the free fatty acids from the precipitated solids through a solid liquid extraction procedure (section 2.3.2). The precipitated solid accounts for 10 - 15% of the dry mass of the original algae used in the WLEP. Traditional solvent extraction techniques extract lipids from the initial total algal biomass. However, the WLEP utilizes hexane

to separate algal lipids that have previously been removed from the algal biomass, which results in a smaller mass of solids for extraction compared to the total cell biomass. Therefore, the WLEP may require less volume of solvent to isolate and collect algal lipids over traditional solvent based lipid extraction processes, further reducing algal lipid extraction costs, as the procedure is scaled up.

3.4. Potential for bio-products from "side" streams

Streams 1 through 3 are considered "side" streams of the WLEP. Streams 1 and 2 can be used for the generation of additional bioproducts. Stream 1 consists of hydrolyzed algal biomass that can be used to generate acetone, butanol, and ethanol via fermentation (Ellis et al., 2012). Stream 2 is an aqueous phase that contains soluble cellular components such as sugars, proteins, and other organic compound that can be used as substrate sources for organisms to generate additional products of value. Utilization of the sugars, proteins, and other components of the algal biomass, in addition to lipids, will help improve the overall economics of algal biofuel production (U.S. DOE, 2010).

4. Conclusions

The wet lipid extraction procedure (WLEP) has been demonstrated to extract 79% of the transesterifiable lipids contained in the wet algal biomass used (84% moisture) via acid and base hydrolysis. Overall, approximately 60% of the transesterifiable lipids within the algal biomass were isolated for conversion to biodiesel. This is achieved without drying the harvested biomass, while potentially helping to reduce the need for organic solvents. Additionally, chlorophyll was precipitated removing, or reducing, chlorophyll contamination of the algal lipid extract. The WLEP provides an approach to algal biomass processing to reduce material and energy costs associated with algal biofuels production.

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CHAPTER 4

EFFECT OF MOISTURE ON THE *IN SITU* TRANSESTERIFICATION OF MICROALGAE FOR BIODIESEL PRODUCTION

1. Introduction

In 2009 petroleum was the primary source of energy in the US, accounting for 37.3% of the total energy flow within the country. Additionally, 72% of the energy generated from petroleum was accounted for within the transportation sector, constituting 94% of the energy used in the transportation sector. [1] Reliance on petroleum fuels at such high rates is unsustainable due to increasing fuel costs, diminishing crude oil reserves, and the environmental consequences that stem from fossil fuel use. [2,3,4,5] These and other problems related to the use of fossil fuels have led to significant interest in finding alternative sources of energy.

One option that has gained interest is the production of biodiesel from renewable sources such as plant oils, animal fats, or microalgae to offset usage of crude oil based diesel. [2,3,4] Microalgae possess advantages over other potential sources of renewable oil in that they; (1) do not shift food resources to energy production; (2) have higher growth rates than land based crops; (3) can be grown in non-arable land using various sources and qualities of water; (4) require little maintenance; (5) can produce high concentrations of intracellular lipids; (6) and are vastly more efficient in converting solar energy into biomass (Table 10). [3,6,7,8,9]

Numerous processes currently exist for the extraction and/or conversion of oils to biodiesel including solvent extraction, use of super-critical solvents, sub-critical water extraction, and *in situ* transesterification. [5,6,10] Of these methods *in situ* transesterification shows promise of both simplifying and reducing the cost of producing biodiesel from microalgal biomass. *In situ* transesterification combines both the lipid extraction and transesterification steps into a single step, eliminating the need for large volumes of organic solvents for oil extraction, which are both costly and in many cases toxic and unstable. [7,8,9] Oil extraction and transesterification are

achieved by contacting dry algal biomass directly with an alcohol and catalyst while heating. Generally methanol is used, due to its availability and low cost. [11] Sulfuric acid is used as the catalyst for the transesterification of both vegetable and algal lipids. [11,12,13] As lipids are extracted from the algal cells, they are simultaneously converted to fatty acid methyl esters (FAME) or biodiesel.

Table 10. Comparison of sources of oil for biodiesel production. [3]

Crop	Oil Yield (L/ha)	Land Area Needed (M ha)[a]	Percent of Existing US Cropping Area[a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil Palm	5950	45	24
Microalgae[b]	136900	2	1.1
Microalgae[c]	58700	4.5	2.5

[a] For meeting 50% of all transport fuel needs of the United States.
[b] 70% oil (by wt) in biomass
[c] 30% oil (by wt) in biomass

In situ transesterification, has certain drawbacks that can limit its effectiveness in converting lipids to FAMEs. [8,14] One requirement is that the algal biomass be dried before transesterification. Three explanations have been commonly presented to describe the inhibitory effect of water on the *in situ* transesterification process. (1) The formation of FAMEs is a reversible reaction, therefore, water can hydrolyze biodiesel back to methanol and free fatty acids, [14] (2) Water contained within the biomass has a tendency to shield lipids from the extracting solvent, preventing lipids from being brought into the reaction, [15] and (3) the acid catalyst can be deactivated due to water competing for available protons in the reaction. [16]

Based on this information, it becomes important to understand and evaluate the extent of this inhibition. Algal biomass moisture content is more critical when considering the amount of energy and cost required to dry large quantities of algal biomass. Understanding how to control the reaction conditions may be advantageous in lessening the impact of water on biodiesel yield. This study examined the inhibitory effect of water on the *in situ* transesterification reaction, and determined changes in biodiesel yield that occurs when altering reaction conditions associated with the *in situ* transesterification method.

2. Materials and Methods

2.1. Reagents and chemicals

Reagents used in this study include ACS grade sulfuric acid from EMD Chemicals (Gibbstown, New Jersey) and methanol obtained from Pharmco-AAPER (Brookfield, CT). HPLC grade hexanes from Fisher Chemicals (Pittsburgh, PA), and. Fatty acid methyl ester (FAME) standards were obtained from Supelco Analytical (Bellfontaine, PA). All macronutrients used for media were laboratory or ACS grade, while micronutrients were technical or laboratory grade.

2.2. Growth and collection of algal biomass

For this study algal biomass was grown in well mixed indoor 15 L bioreactors. The initial inoculum for each of the bioreactors originated from the Logan Lagoons municipal wastewater treatment plant located in Logan, Utah. *Chlorella* and *Scenedesmus* sp. accounted for a majority of the species present in the inoculum. [17] The media in the three bioreactors were mixed using air filtered through Whatman Polyvent 0.2 um filters via spargers, pH was monitored using Sensorex pH probes and maintained at 7.7 with CO_2 addition and measured using Omega PHCN-201 pH controllers, and light was provided by GE Plant and Aquarium Ecolux lights with a total light intensity of approximately 1250 μmol m^2 s^{-1} for a period of 14 hours per day.

Media used for the biomass was a modified form of the SE media, [18] which contained the following macronutrients in units of g/L: 0.85 $NaNO_3$, 0.35 KH_2PO_4, 0.15 $MgSO_4 \cdot 7H_2O$, 0.15 K_2HPO_4, 0.05 $CaCl_2 \cdot 2H_2O$, 0.05 NaCl, and 0.015 $C_6H_8O_7 \cdot Fe \cdot NH_3$. In addition, the following micronutrients were added in units of mg/L: 2.86 H_3BO_3, 1.81 $MnCl_2 \cdot 4H_2O$, 0.22 $ZnSO_4 \cdot 7H_2O$, 0.079 $CuSO_4 \cdot 5H_2O$, and 0.039 $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. Before inoculation, the media was adjusted to a pH of 7.0 using $NaHCO_3$.

All biomass was harvested from the media by centrifugation at 9900xg for 5 minutes. Once harvested, the algal biomass was thoroughly mixed to account for any variation in the biomass between the three reactors. Algal paste was massed into appropriate containers and stored at -80^{o}C until they were to be used.

2.3. Biomass preparation for study

The inhibitory effect of water on the *in situ* transesterification reaction was evaluated using two approaches. The first approach was to directly observe the impact of moisture on biodiesel yield by drying algal biomass to different extents and correlating moisture content to the biodiesel yield. The second approach was to use algal biomass of constant moisture content and vary the *in situ* transesterification reaction parameters to observe changes in biodiesel yield.

A set of five algal samples from the harvested biomass was lyophilized to determine total moisture content of the algal biomass used in this study. A moisture content of 83.88 ±0.75% was calculated based on the mass of the algal biomass before and after freeze drying.

2.4. Effect of moisture on biodiesel yield

Algal biomass was dried by either freeze drying or drying at elevated temperatures. For lyophilization, the centrifuged biomass was frozen at -80^{o}C and placed in a LABCONCO Freezone 4.5 freeze dryer. Drying at elevated temperatures was achieved using an Omegalux (LMF-3550) oven. Algal biomass was subjected to oven temperatures of 65, 85, 105 ^{o}C for 1, 2,

4, 8, 20, or 32 hours. This drying process provided algal biomass with varying amounts of water. For each temperature and drying time the moisture content was measured on a dry weight basis.

2.5. Effect of changing reaction parameters on biodiesel yield

Algal biomass of either constant moisture content (84 wt% water) or lyophilized biomass, were transesterified using the *in situ* transesterification method. Reaction parameters varied were the biomass to acidified methanol ratio (100 mg to 0.5 - 4 mL acidified methanol) and the concentration of sulfuric acid in methanol (1-10% v/v). Biodiesel yields were monitored as these reaction parameters where changed for both the lyophilized algal biomass and wet algal biomass providing a means to directly observe the effect of water and changing reaction parameters on reaction efficiency.

2.6. Biodiesel production from algal biomass via In situ Transesterification.

Conversion of algal lipids to fatty acid methyl esters (FAMEs) was accomplished by *in situ* transesterification. For each sample 100 mg of dry, or equivalent mass of wet or partially dry algae, was placed in a glass test tubes and sealed using PTFE lined screw caps. To the samples 1 mL of a solution containing 5% (v/v) sulfuric acid in methanol was added and mixed. Test tubes were placed in a Hach DRB 200 heat block set to 90^{o}C and allowed to react for a total of 30 minutes with mixing after 15 minutes. After 30 minutes of heating the samples were removed from the heat source and cooled in cold water.

Once the reaction mixture cooled, 5 mL of hexanes was added to the tube, mixed, sealed, and heated to 90^{o}C for 15 minutes to extract FAMEs from the methanol phase into the hexane phase. The tubes were removed from the heat block after 15 minutes and cooled using a cold water bath. Samples were then centrifuged using a bench-top centrifuge to clarify and remove cell debris from the hexane phase.

2.7. Gas chromatography.

FAMEs, in the collected hexane phase, were analyzed using an Agilent 7890A GC equipped with an FID detector. A Restek Stabilwax-DA column (Bellefonte, PA) (30m x 0.32 mm id x 0.25μm film thickness) was used to separate individual FAME compounds. Helium was used as the carrier gas at a constant flow of 2 mL/min. The oven was held at 100^{o}C for 1 minute and then ramped to 235^{o}C at a rate of 10^{o}C/min and held for 10 minutes. The front inlet was operated in splitless mode, with an initial temperature of 100^{o}C for 0.1 minutes and then increased to 235^{o}C at a rate of 720^{o}C/min. Injection volume was set at 1 μL. FID temperature was maintained at 240^{o}C.

Generated FAMEs were quantified by comparing sample peak areas to a linear concentration correlation generated by the serial dilution of a C-8 to C-24 FAME standard mixture from Supelco Analytical. Biodiesel yield was reported as a mass percentage of the algal biomass used based on the total mass of FAMEs generated and the mass of dry algal biomass processed.

3. Results and Discussion

3.1. Effect of moisture content on biodiesel yield

The moisture contents of the algal biomass are presented in Table 11 for each drying temperature and time. Algal biomass was transesterified, and the biodiesel yield was measured by gas chromatography. Figure 14 illustrates biodiesel yields obtained from algal biomass for the various drying times used. Lyophilized algal biomass was transesterified and used as a positive control to provide a maximum biodiesel yield.

The trend shown in Figure 14 illustrated that FAME generation via the *in situ* transesterification reaction increased more rapidly as the drying temperature increased due to more rapid removal of water. When algal biomass was dried at 105^{o}C, FAME yields reached values greater than 90% of the maximum after two hours of drying. At 85^{o}C, FAME yields

reached levels greater than 90% of the maximum by 4 hours of drying, while at 65°C, 6 hours of drying was required. However, additional drying of the biomass did not increase the biodiesel yield.

Table 11. Moisture contents of algal biomass as a function of drying temperature and time. Average values (dry weight basis) are presented with one standard deviation of triplicates.

	105°C	85°C	65°C
Wet (as centrifuged)	81.30 (±0.00%)	88.69 (± 0.00%)	73.89 (±0.00%)
1 Hour	28.55 (±3.88%)	51.30 (±1.63%)	53.61 (±2.09%)
2 Hours	3.81 (±0.97%)	17.25 (±0.41%)	35.84 (± 2.87%)
4 Hours	0.43 (±0.07%)	0.53 (±0.08%)	17.52 (± 2.25%)
8 Hours	0.23 (±0.10%)	0.19 (±0.03%)	2.72 (± 0.03%)
20 Hours	0.06 (±0.01%)	0.05 (±0.01%)	1.77 (± 0.11%)
32 Hours	0.01 (±0.08%)	-0.03 (±0.10%)	1.59 (± 0.04%)
Lyophilized	0.00 (± 0.09%)	0.00% (± 0.02%)	0.00% (± 0.01%)

By correlating algal moisture content to biodiesel yield, as presented in Figure 15, results can be used to predict the amount of drying required to achieve the desired reaction efficiency, thereby minimizing energy usage when drying large quantities of algal biomass. However, prediction of biodiesel yields from algal biomass containing higher than 20% moisture becomes difficult due to the high amount of variability in biodiesel yield. Research conducted by Griffiths et al. [14] observed variability in reaction efficiency with increasing water concentration when using a similar method of FAME production from microalgae. These results show that water interferes with the *in situ* transesterification reaction, and has a variable impact on the reaction once a certain concentration of water has been reached.

Centrifugation is a commonly used method to concentrate and dewater algal biomass. However, centrifugation systems typically achieve solids concentrations of 22%. [19] Therefore, centrifuge systems can harvest algal biomass with a moisture content of approximately 80%.

Based on the data presented in Figure 15, this moisture content would lead to a loss of substantial percentage of potential FAMEs.

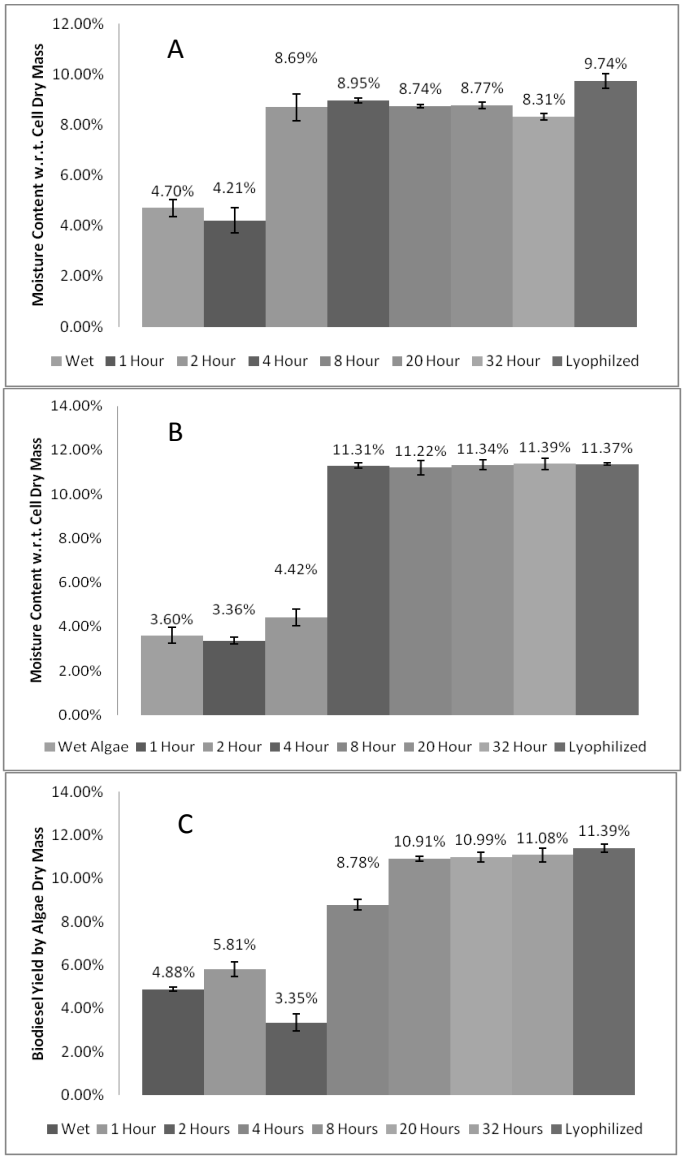

Figure 14. Biodiesel yields from algal biomass as a function of moisture content. A, B, and C refer to biomass dried at temperatures of 105, 85, and 65°C, respectively. Error bars represent one standard deviation.

3.2. Effect of changing reaction parameters on biodiesel yield

Reaction conditions were changed to evaluate their effects on mitigating the inhibitory effects of water on *in situ* transesterification. Reaction conditions that were evaluated included, changing the biomass to acidified methanol volume ratio and/or the concentration of catalyst in

the reaction medium. The generation of FAMEs is reversible and therefore the addition of methanol pushes the equilibrium to the right, or towards FAME production. [20] Increasing the concentration of sulfuric acid may help overcome catalyst deactivation and improve the reaction efficiency.

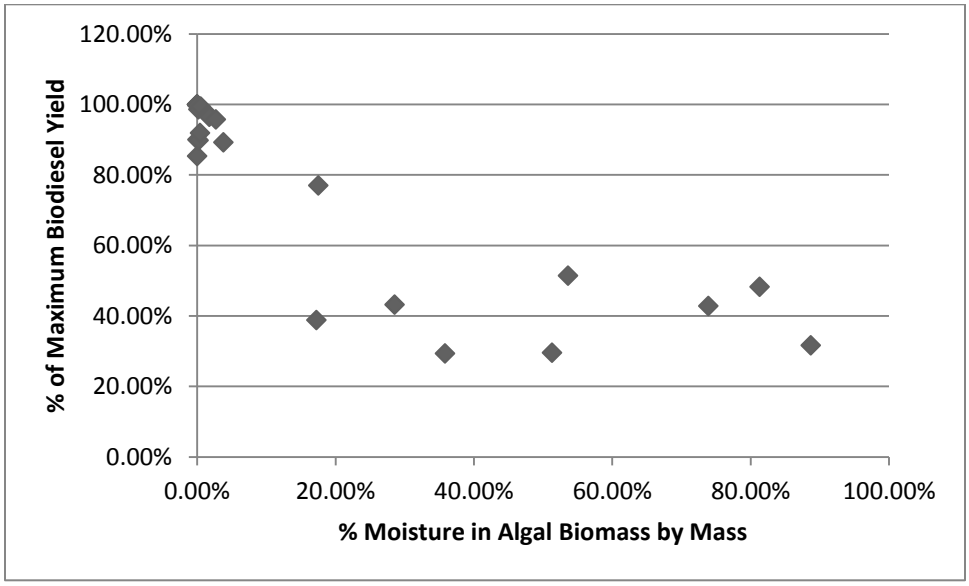

Figure 15. Algal biodiesel yield as a function of algal moisture content. Each data point presented was the average of nine values.

In this study the ratio of algal biomass to acidified methanol solution was varied from 25 to 200 mg dry, or dry mass equivalent, algal biomass per milliliter of acidified methanol solution. The catalyst concentration ranged from 1 to 10% (v/v) sulfuric acid in methanol.

Algal biomass used was either lyophilized or contained 84 wt% water. Lyophilized algal biomass was transesterified to observe the effects of changing the specified parameters without the presence of water, which provided a basis for comparison when wet algal biomass was tested using the same conditions. Figure 16 illustrates results generated from the *in situ* transesterification of lyophilized algal biomass.

Figure 16 shows that increasing methanol to biomass ratio and acid concentration led to improved biodiesel yields from the lyophilized algae. However, increases in acid concentration

greater than 5% did not lead to an improvement in biodiesel yield, indicating that 5% catalyst concentration in methanol is sufficient for complete conversion of lipids to FAMEs within the reaction time provided. Supplying the reaction with increasing amounts of methanol improved the biodiesel yield as well, most likely due to the shift in reaction equilibrium.

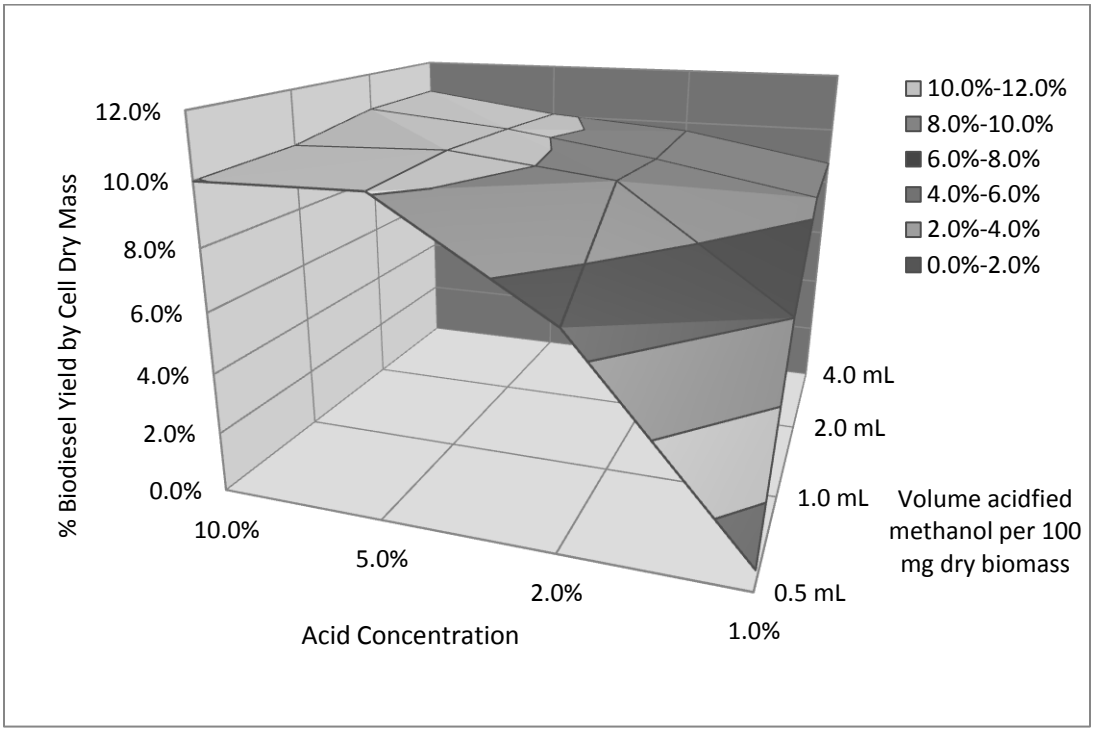

Figure 16. Biodiesel yield as a function of reaction parameters for lyophilized algal biomass.

Results from the *in situ* transesterification of wet algal biomass are presented in Figure 17. Varying acid concentration and the volume of methanol per 100 mg equivalents of wet algal biomass did have an impact on the biodiesel yield. At the most aggressive condition tested, 4 mL acidified methanol per 100 mg biomass and 10% sulfuric acid concentration, the biodiesel yield reached 8.8 wt% FAME with respect to algae dry mass. This represented 81% of the maximum yield observed when using lyophilized biomass.

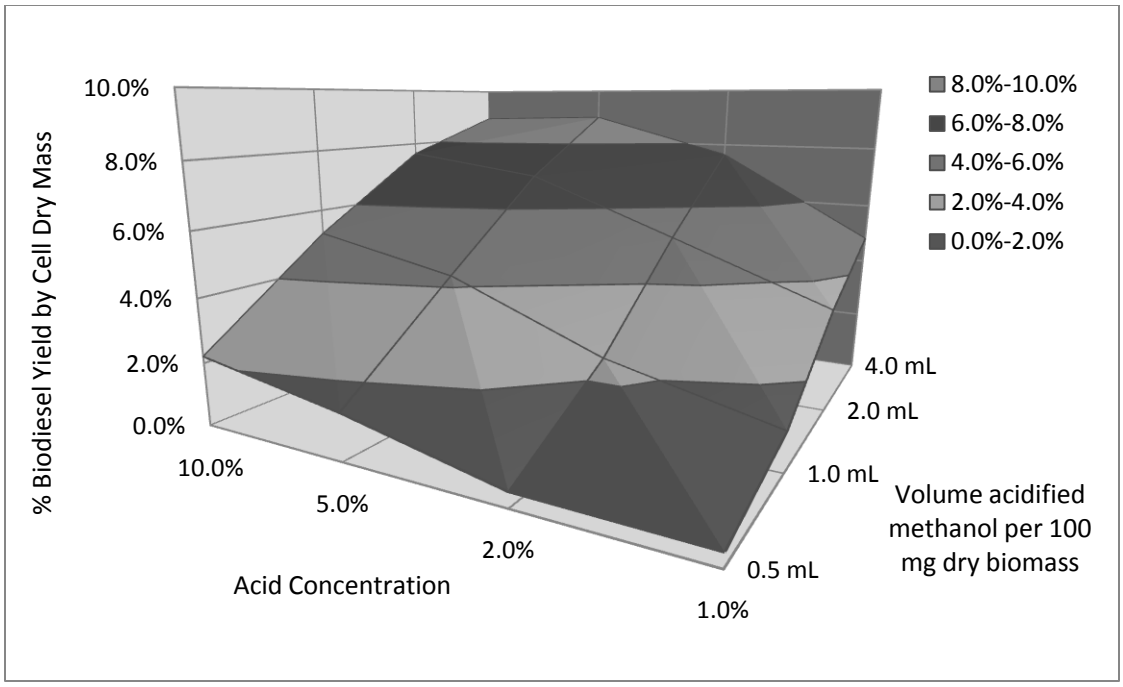

Figure 17. Biodiesel yield with varying reaction parameters for wet algal biomass.

Table 12 presents a direct comparison of data from Figures 16 and 17. At each of the tested reaction conditions the biodiesel yield obtained from wet algal biomass is lower than biodiesel yields obtained from the lyophilized biomass. This difference is most pronounced when using a biomass to acidified methanol ratio of 200 mg biomass per mL solution. When the amount of methanol is increased resulting in a ratio of 25 mg biomass per mL of solution, the difference in biodiesel yield decreases allowing the wet biomass biodiesel yield to reach levels greater than 80% of the maximum. Figure 18 illustrates the differences in FAME yields between the wet and lyophilized biomass for two of the biomass to acidified methanol ratios tested.

These data indicate the potential for overcoming the inhibitory effect of water on the *in situ* transesterification reaction by changing relevant reaction parameters that include methanol to biomass ratio and catalyst concentration. The capability to extract and covert greater than 80% of the transesterifiable lipids present in biomass containing 84% moisture has been demonstrated. It is possible that further increases in the acid concentration and methanol volumes in the reaction could allow for even greater yields.

Table 12. Biodiesel yields for both wet (84 wt% moisture) and lyophilized algal biomass using *in situ* transesterification with varying reaction parameters. Error values represent one standard deviation of triplicates.

mg dry algal biomass/ mL acidified methanol		10.0%	5.0%	2.0%	1.0%
200.0	(dry)	92.5% ±3.61%	93.4% ±4.81%	63.5% ±5.09%	6.4% ±0.83%
	(wet)	20.5% ±2.31%	13.4% ±0.56%	4.2% ±0.28%	3.7% ±0.83%
100.0	(dry)	94.1% ±4.26%	95.6% ±2.04%	90.0% ±1.30%	55.7% ±2.04%
	(wet)	48.2% ±0.00%	39.5% ±1.30%	22.0% ±5.09%	11.3% ±0.56%
50.0	(dry)	99.1% ±11.02%	94.7% ±1.57%	87.6% ±1.85%	78.6% ±2.31%
	(wet)	70.6% ±1.30%	64.6% ±2.50%	47.9% ±1.02%	30.8% ±1.85%
25.0	(dry)	100.0% ±5.00%	93.3% ±3.89%	89.5% ±1.39%	80.7% ±3.15%
	(wet)	81.9% ±2.78%	83.0% ±0.83	70.7% ±2.31%	44.6% ±5.74

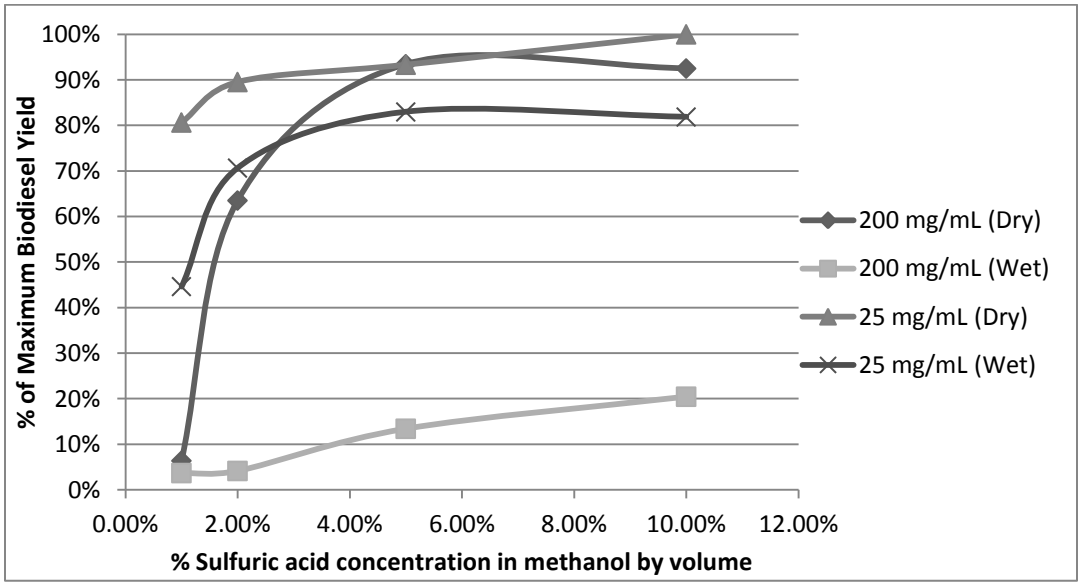

Figure 18. Differences in biodiesel yield from wet and lyophilized algal biomass. Units of mg/mL indicate dry equivalent mass of algal biomass per mL of acidified methanol solution. The difference in biodiesel yield between the dry and wet algal biomass decreases as the acidified methanol to biomass ratio increases (biomass to acidified methanol decreases).

Although an increase in biodiesel yield is possible by increasing methanol and/or sulfuric acid concentration, it requires increasing amounts of raw materials, specifically methanol and sulfuric acid. These additions of chemicals will lead to greater material cost associated with the

production of algal biodiesel. Therefore, a tradeoff is evident between the cost of drying the algal biomass and the cost of materials for improved biodiesel yields. Optimization of the tradeoff is outside the scope of this study and is a topic of further research.

4. Conclusions

This study evaluated the effect of moisture on the *in situ* transesterification of algal biomass as well as method to overcome that effect by changing specific reaction parameters. Moisture has a significant effect on the efficiency of the *in situ* transesterification reaction. Significant reduction in the biodiesel yield when the moisture content exceeds 20 wt% by algae dry mass was observed when algal biomass was dried at three different temperatures. However, it was possible to partially overcome the inhibition by altering the reaction conditions. Reaction efficiency was improved from 40% to 80% when using algal biomass consisting of 16% solids. This was achieved by increasing the volume of methanol in the reaction by a factor of 4 and doubling the sulfuric acid concentration in the reaction medium.

Although the inhibition can be reduced by increasing the amount of alcohol and catalyst present in the reaction, the addition of these materials adds to the processing cost of producing biodiesel. Therefore, an optimization is required between the cost of additional raw materials, in methanol and sulfuric acid, versus the increase in biodiesel yield. These issues will be critical in evaluating and implementing larger scale biodiesel production facilities in the future.

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CHAPTER 5

SUMMARY

The coupling of wastewater remediation to the production of renewable fuels is a promising technology. However, a number hurdles have prevented the large scale production, harvesting, and processing of algal biomass into biofuels. Of these hurdles the requirement that algal biomass be dried prior to lipid extraction, for biodiesel production, has been a significant energetic hurdle. In addition, traditional lipid extraction and/or conversion methods applied to algal biomass have resulted in the biodiesel containing contaminants that can require costly purification processes.

Attempting to process wet algal biomass leads to severe decreases in the yield of biodiesel from algal biomass, reflected in the data presented in Figure 15. Such losses in biodiesel yield when using the *in situ* method of transesterification reduces the feasibility of economically processing algal biomass to biodiesel. Therefore, further research conducted leading to the development of the wet lipid extraction procedure (WLEP), which has been demonstrated to extract up to 79% of the transesterifiable lipids from wet algal biomass and isolate up to 60% of the total algal lipids for biodiesel production.

During the WLEP chlorophyll is precipitated and isolated as a solid phase, allowing for the isolation of algal lipids from wet algal biomass while simultaneously removing or reducing chlorophyll contamination. This provides a means to convert the algal lipids to biodiesel with removed or reduced chlorophyll contamination. This is a problem many traditional algal biodiesel producing processes suffer from, which the WLEP has been able to address.

Furthermore, as shown in Figure 11, the WLEP generates four streams that are removed from the procedure. These are the residual biomass, aqueous phase, solid phase precipitate, and extracted lipid residue streams. The extracted lipids are converted to biodiesel; however, the other three streams can be used for the production of additional bioproducts and biofuels. The residual

biomass stream consists of algal biomass digested with acid and base making it readily available for microbial digestion or fermentation processes. The aqueous phase is composed of soluble cell components such as sugars, proteins, glycerol (derived from the hydrolysis of complex lipids), and other organic compounds. The aqueous phase can be used as a liquid media for the growth of microbes capable of generating economically valuable bioproducts. The final solid phase precipitate contains chlorophyll, which may be refined to generate either purified chlorophyll or porphyrin products

Research presented in this thesis illustrates the impact of water on the *in situ* method of transesterification for production of algal biodiesel. Based on the severe impact of water on the conversion efficiency of lipids to biodiesel using the *in situ* transesterification method, a novel procedure was developed known as the wet lipid extraction procedure (WLEP). The WLEP is capable of extracting and isolating up to 60% for biodiesel production, while simultaneously precipitating chlorophyll providing a means to remove or reduce chlorophyll contamination of the resulting biodiesel. In addition, the WLEP generates side streams that can be utilized as raw material for the production of additional bio-products, thus aiding the overall economics of generating biofuels and bioproducts from algal biomass.

The capability of the developed WLEP to extract lipids from wet algal biomass, to reduce/remove chlorophyll contamination, to potentially reduce organic solvent demand, and to generate feedstocks for high-value bioproducts presents opportunities to reduce costs of scaling up algal lipid extraction for biodiesel production. These advances may make it more feasible to realistically scale up the production algal biomass via the remediation of wastewater and utilize that biomass to generate bioproducts and biofuels such as biodiesel at scale.

APPENDICIES

APPENDIX A

PROVISIONAL PATENT APPLICATION: 61/551, 049

METHOD OF LIPID EXTRACTION[2]

TECHNICAL FIELD

[0001] The present disclosure relates to lipid extraction, more specifically, to lipid extraction from algal biomass for biodiesel production.

BACKGROUND

[0002] The production of biodiesel from various biological feed stocks, such as vegetable oil, animal fats, halophytes, and algae has been explored in an effort to enable alternative fuel sources. Extraction of the oil from biological feed stocks may be undertaken by various conventional methods depending on the feed stock. However, improved methods for extracting the oil from algae are needed for commercial viability and/or feasibility to be established.

SUMMARY

[0003] Typically, algae as a biodiesel feed stock is dried prior to processing. However the energy costs of harvesting and then drying algae from, for example, waste ponds, are substantial. What's more, a drying step is time intensive. The processes described herein allow for lipid extraction from algal biomass in wet form, which can significantly reduce the overall production costs

[2] Adapted and formatted by Ryan Brady USU Associate General Counsel

of biodiesel from algae. This method also eliminates or drastically reduces the pigments carried through conventional processes, which can taint the end product biodiesel if purification steps are not taken. For example, the presence of chlorophyll and other pigments requires complicated purification steps to generate useable biodiesel; generally vacuum distillation. Such additional steps may be avoided if the pigments are reduced.

[0004] The present disclosure in aspects and embodiments addresses these various needs and problems by providing methods for extracting lipids from algae, the methods comprising hydrolyzing a slurry comprising algae and water by adding an acidic hydrolyzing agent to yield an acidic slurry, hydrolyzing the acidic slurry by adding a basic hydrolyzing agent to yield a basic slurry, separating an aqueous phase from biomass in the basic slurry, forming a precipitate in the aqueous phase, and extracting free fatty acids from the precipitate.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] Figure 1 illustrates an exemplary method of producing biodiesel.

[0006] Figure 2 illustrates the precipitation of algal pigments that occurs using an exemplary method.

DETAILED DESCRIPTION

[0007] The present disclosure covers methods, compositions, reagents, and kits for an improved method of lipid extraction from algal biomass. In the

following description, numerous specific details are provided for a thorough understanding of specific preferred embodiments. However, those skilled in the art will recognize that embodiments can be practiced without one or more of the specific details, or with other methods, components, materials, etc. In some cases, well-known structures, materials, or operations are not shown or described in detail in order to avoid obscuring aspects of the preferred embodiments. Furthermore, the described features, structures, or characteristics may be combined in any suitable manner in a variety of alternative embodiments. Thus, the following more detailed description of the embodiments of the present invention, as illustrated in some aspects in the drawings, is not intended to limit the scope of the invention, but is merely representative of the various embodiments of the invention.

[0008] In this specification and the claims that follow, singular forms such as “a,” “an,” and “the” include plural forms unless the content clearly dictates otherwise. All ranges disclosed herein include, unless specifically indicated, all endpoints and intermediate values. In addition, “optional” or “optionally” refer, for example, to instances in which subsequently described circumstance may or may not occur, and include instances in which the circumstance occurs and instances in which the circumstance does not occur. The terms “one or more” and “at least one” refer, for example, to instances in which one of the subsequently described circumstances occurs, and to instances in which more than one of the subsequently described circumstances occurs.

[0009] In some embodiments, the methods may include the following steps: (1) acid hydrolysis, (2) base hydrolysis, (3) biomass and aqueous phase separation, (4) precipitate formation, (5) free fatty acid extraction, and optionally (6) biodiesel production. Figure 1 illustrates a flow diagram of an exemplary method.

[0010] **Feed Stock**

[0011] As a feed stock, any suitable algae may be used. In embodiments, algae that produces high lipid amounts may be preferred. In many embodiments, algae produced on waste water may be used. The algae may be lyophilized, dried, in a slurry, or in a paste (with for example 10-15% solid content).

[0012] After identification of a feed stock source or sources, the algae may be formed into a slurry, for example, by adding water, adding dried or lyophilized algae, or by partially drying, so that it has a solid content of about 1-40%, such as about 4-25%, about 5-15%, about 7-12%, or about 10%.

[0013] The various steps to the process, according to some embodiments, is described in more detail below. The methods described herein may be accomplished in batch processes or continuous processes.

[0014] **(1) Acid Hydrolysis**

[0015] To degrade the algal cells (or other cells present), to bring cellular components into solution, and to break down complex lipids to free fatty acids, the slurry of water and algae described above may be optionally heated and

hydrolyzed with at least one acidic hydrolyzing agent. These complex lipids may include, for example, triacylglycerols (TAGs), glycolipids, etc. In addition to degrading algal cells and complex lipids, the acidic environment created by addition of the hydrolyzing agent removes the magnesium from the chlorophyll molecules (magnesium can otherwise be an undesirable contaminant in end-product biodiesel).

[0016] When heated, the slurry may reach temperatures of from about 1-200ºC, such as about 20-100ºC, about 50-95ºC, or about 90ºC. When temperatures above 100ºC, or the boiling point of the solution are used, an apparatus capable of withstanding pressures above atmospheric pressure may be employed. In some embodiments, depending on the type of algae, the type and concentration of acid used for hydrolysis, the outside temperature conditions, the permissible reaction time, and the conditions of the slurry, heating may be omitted. Heating may occur prior to, during, or after addition of a hydrolyzing agent.

[0017] In addition, the slurry may be optionally mixed either continuously or intermittently. Alternatively, a hydrolysis reaction vessel may be configured to mix the slurry by convection as the mixture is heated.

[0018] Acid hydrolysis may be permitted to take place for a suitable period of time depending on the temperature of the slurry and the concentration of the hydrolyzing agent. For example, the reaction may take place for up to 72 hours, such as from about 12-24 hours. If the slurry is heated, then hydrolysis may

occur at a faster rate, such as from about 15-120 minutes, 30-90 minutes, or about 30 minutes.

[0019] Hydrolysis of the algal cells may be achieved by adding to the slurry a hydrolyzing agent, such as an acid. Any suitable hydrolyzing agent, or combination of agents, capable of lysing the cells and breaking down complex lipids may be used. Exemplary hydrolyzing acids may include strong acids, mineral acids, or organic acids, such as sulfuric, hydrochloric, phosphoric, or nitric acid. These acids are all capable of accomplishing the goals stated above. When using an acid, the pH of the slurry should be less than 7, such as from about 1-6, about 1.5-4, or about 2-2.5.

[0020] In addition to strong acids this digestion may also be accomplished using enzymes alone or in combination with acids that can break down plant material. However, any such enzymes or enzyme/acid combinations would also be capable of breaking down the complex lipids to free fatty acids.

[0021] In some embodiments, the acid or enzymes, or a combination thereof, may be mixed with water to form a hydrolyzing solution. However, in other embodiments, the hydrolyzing agent may be directly added to the slurry.

[0022] **(2) Base Hydrolysis**

[0023] After the initial hydrolysis, a secondary base hydrolysis may be performed to digest and break down any remaining whole algae cells; hydrolyze

any remaining complex lipids and bring those lipids into solution; convert all free fatty acids to their salt form, or soaps; and to break chlorophyll molecules apart.

[0024] In this secondary hydrolysis, the biomass in the slurry is mixed with a basic hydrolyzing agent to yield a pH of greater than 7, such as about 8-14, about 11-13, or about 12-12.5. Any suitable base may be used to increase in pH, for example, sodium hydroxide, or other strong base, such as potassium hydroxide may be used. Temperature, time, and pH may be varied to achieve more efficient digestion.

[0025] This basic slurry may be optionally heated. When heated, the slurry may reach temperatures of from about 1-200ºC, such as about 20-100ºC, about 50-95ºC, or about 90ºC. When temperatures above 100ºC, or the boiling point of the solution are used, an apparatus capable of withstanding pressures above atmospheric pressure may be employed. In some embodiments, depending on the type of algae, the type and concentration of acid used for hydrolysis, the outside temperature conditions, the permissible reaction time, and the conditions of the slurry, heating may be omitted. Heating may occur prior to, during, or after addition of a hydrolyzing agent.

[0026] In addition, the basic slurry may be optionally mixed either continuously or intermittently. Alternatively, a hydrolysis reaction vessel may be configured to mix the slurry by convection as the mixture is heated.

[0027] Basic hydrolysis may be permitted to take place for a suitable period of time depending on the temperature of the slurry and the concentration

of the hydrolyzing agent. For example, the reaction may take place for up to 72 hours, such as from about 12-24 hours. If the slurry is heated, then hydrolysis may occur at a faster rate, such as from about 15-120 minutes, 30-90 minutes, or about 30 minutes.

[0028] During this basic hydrolysis, chlorophyll is hydrolyzed to the porphyrin head and phytol side chain.

[0029] **(3) Biomass and Aqueous Phase Separation**

[0030] Under the condition of elevated pH, the biomass may be separated from the aqueous solution. This separation is performed while the pH remains high to keep the lipids in their soap form so that they are more soluble in water, thereby remaining in the water phase. Once the separation is complete, the water phase is kept separate and the remaining biomass may be optionally washed with water to help remove any residual soap molecules. This wash water may also be collected along with the original liquid phase. Once the biomass is washed it may be removed from the process.

[0031] The liquid phase now contains the recovered lipids in soap form, Porphyrin salts, and any other soluble cellular components. Much of the hydrophobic cellular components are potentially removed with the biomass, for example, pigments such as carotenoids.

[0032] Any suitable separation technique may be used to separate the liquid (aqueous) phase form the biomass. For example, centrifugation, gravity

sedimentation, filtration, or any other form of solid/liquid separation may be employed.

[0033] **(4) Precipitate Formation**

[0034] After the biomass is removed, the pH of the collected liquid may be neutralized/reduced to form a precipitate. This may be accomplished by the addition of an acid to the solution, such as at least one strong acid or mineral acid, for example, sulfuric, hydrochloric, phosphoric, or nitric acid. Addition of a suitable acid is performed until a green precipitate is formed. The green precipitate may contain, or may be, the Porphyrin heads as they are converted from their salt forms. It may also contain proteins and other cellular components that are coming out of solution.

[0035] The pH may be reduced to a pH of about 7 or less, such as about 4-6.9. This lower pH also converts the soap in the liquid to free fatty acids. As the precipitate forms the fatty acids associate with the solid phase and come out of solution. Once the precipitate has formed, the solid and liquid phases may be separated. Any suitable separation method may be employed, such as centrifugation, gravity sedimentation, filtration, or any other form of solid/liquid separation. The liquid phase may be removed from the process. The collected solid phase may then be processed further. Optionally, the precipitate may be lyophilized or dried, which may result in nearly complete extraction of the lipids during extraction.

[0036] **(5) Free Fatty Extraction and solvent recycle**

[0037] To extract the free fatty acids, an organic solvent may be added to the solid phase resulting from the previous step. The solid phase may be mixed with the solvent and then optionally heated to facilitate fatty acid extraction from the solid phase.

[0038] When heated, the mixture of solid phase and solvent may reach temperatures of from about 1-200ºC, such as about 20-100ºC, about 50-9 ºC, or about 90ºC. When temperatures above 100ºC, or the boiling point of the solution are used, an apparatus capable of withstanding pressures above atmospheric pressure may be employed. In some embodiments, heating may be omitted. Heating may occur prior to, during, or after the mixture of solid phase and solvent is formed. In addition, the mixture may be optionally mixed either continuously or intermittently.

[0039] The extraction process may be permitted to take place for a suitable period of time depending on the temperature of the mixture. For example, the extraction may take place for up to 72 hours, such as from about 12-24 hours. If the mixture is heated, then extraction may occur at a faster rate, such as from about 15-120 minutes, 30-90 minutes, or about 30 minutes.

[0040] During this time the free fatty acids associated with the solid are extracted into the organic phase. Suitable solvents include non-polar solvents, such as hexane, chloroform, pentane, tetrahydrofuran, and mixtures thereof (for example a 1:1:1 ratio of chloroform, tetrahydrofuran, and hexane). Other suitable solid-liquid extraction methods and unit operations may be used.

[0041] Once the free fatty acids are extracted, the solid phase may be removed from the process and the organic phase may be vaporized and recycled. What remains after the organic phase is vaporized is a residue consisting of mostly the free fatty acids or algal lipids/oil. This algal oil may then optionally be processed into biodiesel.

[0042] **(6) Biodiesel production from algal oil and collection**

[0043] The algal oil collected in the previous step may be converted to biodiesel by esterification. This is done by the addition of a strong acid catalyst and an alcohol to the oil. With the addition of heat, the alcohol and catalyst will work to convert the free fatty acids to alkyl esters, also known as biodiesel. Generally this may be done using Sulfuric acid and Methanol, resulting in fatty acid methyl esters or F.A.M.E.s. Once the FAMEs are generated via the esterification reaction, they may be extracted from the reaction mixture using an organic solvent, such as Hexane, and further purified to useable biodiesel. In addition to this method of conversion there are a number of methods that can also be used. However, this method has shown the most promise in terms of being cost effective in conversion of lipids to biodiesel.

[0044] In some embodiments, the steps outlined above may be further simplified and/or combined. For example, in some embodiments, the algal cells may be lysed by any suitable method, including, but not limited to acid hydrolysis. Other methods may include mechanical lysing, such as smashing, shearing, crushing, and grinding; sonication, freezing and thawing, heating, the addition of

enzymes or chemically lysing agents. After an initial lysing of the algal cells, the pH is raised as described above in base hydrolysis to form soap from free fatty acids. The resulting aqueous phase which include the soaps in solution is removed, and then a precipitate containing the free fatty acids is formed by lowering the pH as described above in precipitate formation. The lipids may then be extracted by a suitable method, such as those described above.

[0045] The following examples are illustrative only and are not intended to limit the disclosure in any way.

EXAMPLES

[0046] Example 1: Acid Hydrolysis

[0047] To a glass test tube 100 mg of lyophilized algal biomass was added. One mL of a 1 Molar Sulfuric acid solution is added to the test tube and the test tube was then sealed using a PTFE lined screw cap and gently mixed to create a homogenous slurry. This slurry was then placed in a Hach DRB-200 heat block pre-heated to 90^{o}C. This slurry is allowed to digest for 30 minutes with mixing at the 15 minute mark.

[0048] Example 2: Base Hydrolysis

[0049] Once the first 30 minute digestion period of Example 1 was complete, the test tube was removed from the heat source and 0.75 mL of a 5 Molar Sodium Hydroxide solution was added to the test tube. The test tube was

immediately resealed and returned to the heat source for 30 minutes. Mixing at 15 minutes was again provided.

[0050] Example 3: Biomass Removal

[0051] Once the base hydrolysis step of Example 2 was complete, the test tube was removed from the heat source and allowed to cool in a cold water bath. Once cooled the test slurry was centrifuged using a Fisher Scientific Centrific Model 228 centrifuge. The upper aqueous phase was removed and collected in a separate test tube. To the remaining biomass 1 mL of deionized water as added and vigorously mixed. The slurry was re-centrifuged, and the liquid phase collected and added to the previously collected liquid phase. The biomass was then removed from the process.

[0052] Example 4: Precipitate Formation

[0053] To the collected liquid phase of Example 3, 1.5 mL of a 2 Molar Sulfuric Acid Solution was added, or until a green precipitate was formed. After mixing the liquid became a solid-liquid slurry. This mixture was centrifuged and the upper aqueous phase was removed from the process and the solids were further processed.

[0054] Example 5: Free Fatty Acid Extraction

[0055] Five milliliters of Hexane was added to the collected precipitate of Example 4, which was sealed using a PTFE lined screw cap, and vigorously mixed. The test tube was then placed in the Hach DRB-200 heat block, pre-

heated to 90^{o}C. Extraction of the free fatty acids into the Hexane phase was allowed to continue at 90^{o}C. After a time duration of 15 minutes at 90^{o}C was completed, the test tube was centrifuged to pellet the solids and to allow for the collection of the solvent phase, which as transferred to another test tube. Hexane was allowed to vaporize via gentle heating within the test tube leaving behind the free fatty acid residue.

[0056] Example 6: Fatty Acid Esterification to Biodiesel

[0057] To the residue of Example 5, 1 mL of a 5% (v/v) solution of Sulfuric acid in Methanol was added. This test tube was sealed using a PTFE lined screw cap and the test tube was heated to 90^{o}C for 30 minutes in a Hach DRB-200 heat block. After 30 minutes the test tube was allowed to cool. Upon cooling 5 mL of Hexane was added to the reaction mixture and the test tube was re-sealed and heated again for 15 minutes at 90^{o}C. FAMEs were extracted into the Hexane phase, which were collected and analyzed for biodiesel content using gas chromatography, or another analytical technique or instrument.

[0058] Example 7: Production Efficiency of Water-Based Lipid Extraction

[0059] To test efficiency and the efficacy of heating, the outputs of biodiesel produced according to the methods described herein were tested and compared with a control. Samples were prepared according to the processes described above in Example 1-6, with the exception of heat not being added during the various process steps.

[0060] The findings are summarized in the data table set forth below.

Table A1. Data presenting proof of concept for wet lipid extraction procedure

	mg FAME:	**Standard Deviation: (mg)**	**% of Maximum:**
FAMEs from *in situ* TE:	11.77	0.35	100%
Total FAME Collected:	10.95	1.75	93.02%
FAME in Organic Phase:	*3.11*	*0.53*	*26.43%*
FAME in precipitate:	*5.45*	*1.35*	*46.31%*
FAME in water phase:	*0.14*	*0.01*	*1.23%*
FAME in residual biomass:	*2.24*	*0.25*	*19.04%*

[0061] "FAME(s)" is the contraction for fatty acid methyl ester(s) also known as biodiesel. FAMEs were quantified using gas chromatography. An Agilent 7890-A GC system equipped with a FID detector was used for this purpose.

[0062] "In situ TE" refers to a method of transesterification (in situ transesterification) by which dried algal biomass is directly contacted and subjected to, in this case, Sulfuric acid, Methanol, and heat. This process simultaneously extracts and converts lipids present in the algal biomass to FAMEs or biodiesel. In situ Transesterification is the method favored, throughout the literature, to measure the biodiesel potential for various types of biomass. This method is considered the control and is assumed to completely convert all present lipids in the algal biomass to FAMEs. Each intermediate collected throughout the process was subjected to this method of FAME production to

convert lipids present and quantified by gas chromatography as previously stated.

[0063] “Total FAME collected” refers to the sum of FAMEs measured from each intermediate step throughout the process described in this disclosure. This sum is based on averages of three samples, from within the same batch of algal biomass.

[0064] “FAME in Organic Phase” refers to the quantity of FAME collected in the residue remaining after the organic solvent was vaporized.

[0065] “FAME in precipitate” refers to the quantity of transesterifiable/esterifiable lipids remaining in the precipitated solid phase, formed in the base neutralization step, after being extracted using the organic solvent and heat.

[0066] “FAME in water phase” refers to the quantity of transesterifiable/esterifiable lipids remaining in the aqueous phase after removing the precipitated solid phase.

[0067] “FAME in residual biomass” refers to the quantity of transesterifiable/esterifiable lipids remaining in the residual biomass after both hydrolysis steps.

[0068] Example 8: Pigment Precipitation

[0069] The process outlined in Examples 1-4 was performed on a sample. The resulting precipitate was freeze dried and then re-dissolved in 5 M sodium hydroxide. The resulting solution was run through a Shimadzu UV-1800 UV Spectrophotometer That slide shows absorption data from a Shimadzu UV-1800 UV Spectrophotometer, which measures the absorbance from 300 nm to 900 nm. The results are shown in Figure 2. The “blank,” or lower line along the bottom, refers to plain 5 M Sodium Hydroxide; and the “sample” refers to the re-dissolved precipitate. The data developed demonstrate that pigments are precipitating, a desirable property since pigments can be an undesirable impurity in biodiesel.

[0070] It will be appreciated that various of the above-disclosed and other features and functions, or alternatives thereof, may be desirably combined into many other different systems or applications. Also, various presently unforeseen or unanticipated alternatives, modifications, variations or improvements therein may be subsequently made by those skilled in the art, and are also intended to be encompassed by the following claims.

WHAT IS CLAIMED IS:

1. A method of extracting lipids from wet algae, the method comprising:

hydrolyzing a slurry comprising algae and water by adding an acidic hydrolyzing agent to yield an acidic slurry,

hydrolyzing the acidic slurry by adding a basic hydrolyzing agent to yield a basic slurry,

separating an aqueous phase from biomass in the basic slurry,

forming a precipitate in the aqueous phase, and

extracting free fatty acids from the precipitate.

2. The method of claim 1, wherein the slurry has a solid content of about 4-25%.

3. The method of claim 1, wherein the acidic hydrolyzing agent is selected from the group consisting of a strong acid, a mineral acid, sulfuric acid, hydrochloric acid, phosphoric acid, and nitric acid.

4. The method of claim 1, wherein the acidic slurry has a pH of from about 1.5-4.

5. The method of claim 1, wherein the acidic hydrolyzing agent degrades the algae and breaks down complex lipids to free fatty acids.

6. The method of claim 1, wherein the acidic hydrolyzing agent removes magnesium from algal chlorophyll molecules.

7. The method of claim 1, wherein the acidic slurry is heated to a temperature of from about 50-95 ºC.

8. The method of claim 1, wherein the basic hydrolyzing agent is selected from the group consisting of a strong base, sodium hydroxide, and potassium hydroxide.

9. The method of claim 1, wherein the basic slurry has a pH of from about 8-14.

10. The method of claim 1, wherein the basic hydrolyzing agent converts free fatty acids from the algae to soap.

11. The method of claim 1, wherein the basic slurry is heated to a temperature of from about 50-95 ºC.

12. The method of claim 1, wherein separating the aqueous phase from the biomass in the basic slurry comprises washing separated biomass.

13. The method of claim 1, wherein forming the precipitate in the aqueous phase comprises lowering the pH to about 4-6.9.

14. The method of claim 1, wherein extracting the free fatty acids from the precipitate comprises:

removing a solid phase containing free fatty acids that results from lowering the pH of the aqueous phase; and

mixing the solid phase with a solvent to extract the free fatty acids from the solid phase.

15. The method of claim 14, wherein the solvent is selected from the group consisting of non-polar solvents, hexane, chloroform, pentane, and tetrahydrofuran.

16. A method of producing biodiesel from algae, the method comprising:

hydrolyzing a slurry comprising algae and water by adding an acidic hydrolyzing agent to yield an acidic slurry,

hydrolyzing the acidic slurry by adding a basic hydrolyzing agent to yield a basic slurry,

separating an aqueous phase from biomass in the basic slurry,

forming a precipitate in the aqueous phase, and

extracting free fatty acids from the precipitate, and

converting the extracted free fatty acids to biodiesel by esterification.

17. A method of extracting lipids from algae, the method comprising:

lysing algal cells to form free fatty acids in an aqueous solution;

transforming the free fatty acids to soap in the aqueous solution by increasing the pH;

precipitating the free fatty acids out of the aqueous solution; and

extracting the precipitated fatty acids.

18. The method of claim 17, further comprising converting the extracted free fatty acids to biodiesel by esterification.

ABSTRACT OF THE DISCLOSURE

A method of extracting lipids from wet algae, the method includes hydrolyzing a slurry comprising algae and water by adding an acidic hydrolyzing agent to yield an acidic slurry, hydrolyzing the acidic slurry by adding a basic hydrolyzing agent to yield a basic slurry, separating an aqueous phase from biomass in the basic slurry, forming a precipitate in the aqueous phase, and extracting free fatty acids from the precipitate.

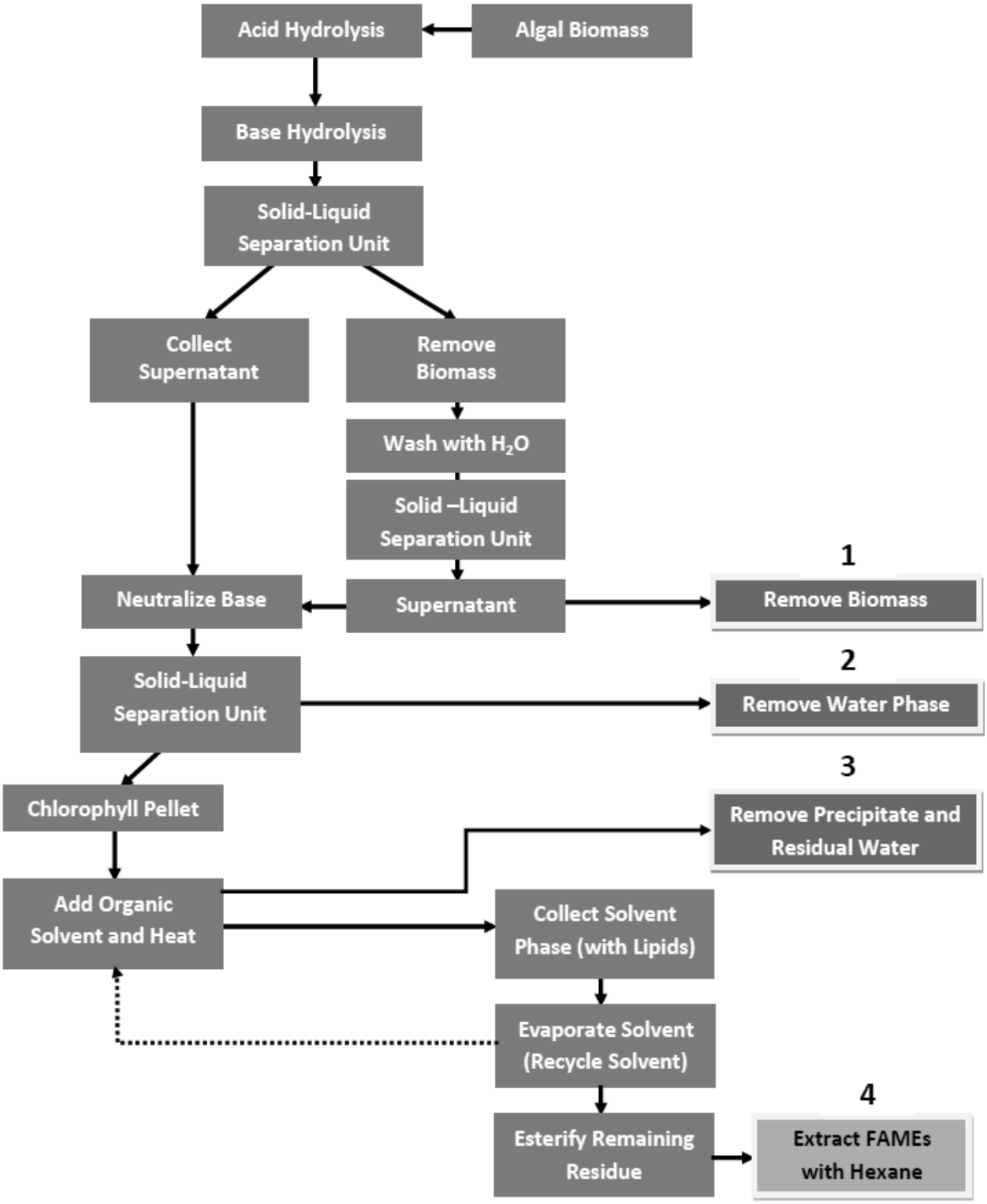

Figure A1. Flow diagram of wet lipid extraction procedure.

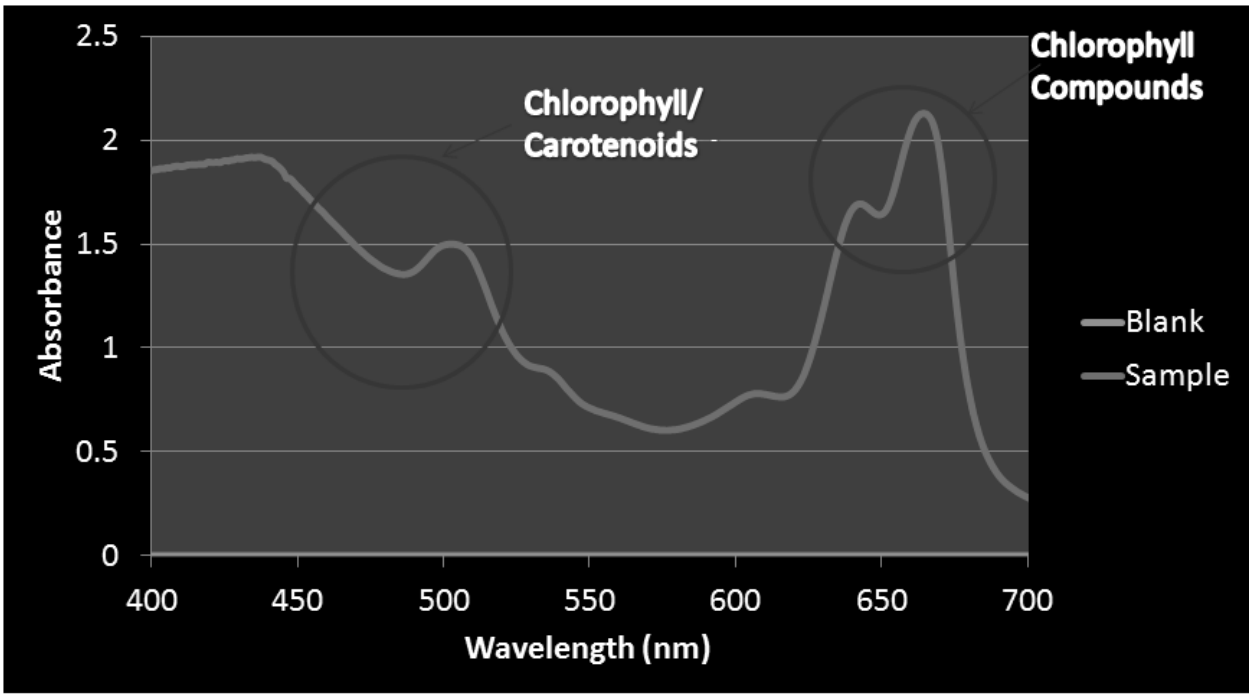

Figure A2. Spectra of solid phase precipitate

APPENDIX B

LIFE CYCLE ANALYSIS REPORT

1. Introduction:

1.1. Logan Utah Open Lagoon Wastewater Treatment Facility

The Logan Lagoons wastewater treatment plant treats municipal wastewater generated from the City of Logan and surrounding areas. Wastewater entering the lagoon system undergoes primary and secondary treatment [1]. However, tertiary treatment is not possible with the system's current configuration. In order to meet regulations on the amount of nitrogen and phosphorous the current lagoons system will have to be retro-fitted to achieve tertiary treatment of the wastewater.

Installation and operation of a mechanical or chemical treatment plant brings significant capital and operation costs [2]. An alternative to implementation of a mechanical or chemical treatment system is to make use of naturally occurring microalgae within the Logan lagoons system.

Microalgae assimilate nitrogen and phosphorous as part of the biomass, sequestering it from the wastewater. By capturing or harvesting the microalgae before they leave the lagoon system, it is possible to remove nitrogen and phosphorous from the wastewater, achieving tertiary treatment of the wastewater [1]. By harvesting and removing the microalgae, containing the excess nutrients, not only is the wastewater being remediated, but this allows for the collection of significant amounts of algal biomass that can be used for the production of biofuels and bioproducts.

1.2. Rotating Algal Biofilm Reactor System versus Traditional Raceway Ponds

Growth and harvesting of algal biomass has been considered a significant hurdle in the production and processing of large quantities of algal biomass [3,4]. Two traditional methods are used for the growth of algal biomass, closed and open systems [5–7]. Closed systems are based on enclosed bioreactors within which algal biomass is grown with tightly controlled conditions [8]. The use of controlled conditions and optimized environment allows for high biomass and lipid

productivities [9]. However, they are considered costly and due to their need for controlled environments and their construction [10].

2. Scope of this Study

This study has multiple goals and varying scopes of analysis. The first focus is to analyze the growth and production of algal biomass using two systems, the traditional raceway and the RABR growth and harvesting systems. These systems were analyzed based on their areal productivity per unit area (4.3 m^2). Parameters studied were energy input to each system to generate 1 kg of biodiesel and the resulting generation of greenhouse gases based on the energy consumed.

In addition to the energy consumption and greenhouse gas emissions generated from each production system, the raceway and the RABR systems' ability to remove nutrients from wastewater was assessed. This assessment also considered the addition of fertilizers to the wastewater in order to achieve maximum nutrient removal, based on stoichiometric needs. It is well known that the addition of fertilizer adds significant energy demand in that the production of fertilizers requires large amounts of energy and generate greenhouse gases themselves [11]. These considerations were also accounted for in this analysis.

Upon producing and collecting the algal biomass it must be processed to generate the various biofuels discussed. Traditionally algal biomass is either dried or processed wet using a solvent based lipid extraction process and the residual lipid extracted algae is sent to an anaerobic reactor for digestion for the production of methane gas [4,12,13]. However, algal biomass harvested in this study is processed using a wet lipid extraction process (WLEP). This procedure is capable of utilizing algal biomass harvested at 15% solids, extract lipids for biodiesel production directly from the wet biomass, and generate feedstock material for the production of acetone, butanol, ethanol, and biomethane. A life cycle analysis was performed for the WLEP and was compared to typical algal biofuel production analyses performed in literature.

The final scope of this study involved the entire Logan lagoons wastewater treatment

facility's capability of producing algal biomass using the RABR growth and harvesting system. A net energy analysis was performed on the production of algal biomass and the biofuels derived from the algal biomass. These biofuels included biodiesel (via WLEP), biomethane (via Anaerobic Digestion), acetone, butanol, and ethanol (via ABE fermentation). The net energy analysis took into account the energy consumed by the RABR growth and harvesting system and the energy generated via the biofuels, based on their heats of combustion. The difference in the energy consumed and generated provides an indication of the energy available to produce the biofuels.

3. Nutrient Removal in Raceway and RABR systems

3.1. Parameters used for analysis

Removal of nutrients such as inorganic nitrogen and phosphorous from wastewater using microalgae is a concept that has been proposed by multiple authors [11,14,15]. This portion of the analysis focused on the capability of each system to remove nutrients from the wastewater. For both systems a unit volume of 1 m^3 of wastewater was used as the basis.

In order to achieve maximum removal of nutrients the concentrations need to be balanced according to the stoichiometry of the algal biomass. As described in this report, the concentration of nitrogen and phosphorous in the wastewater reaching the algal growth systems is approximately 7.8 mg/L N and 4.5 mg/L. This corresponds to a 3.8 to 1 (N: P) molar ratio. Stoichiometry of algal biomass was based on the Redfield expression $C_{106}H_{263}O_{110}N_{16}P_1$ [16]. Based on this expression it is clear the wastewater is nitrogen limited for supporting algal growth. Without the addition of nitrogen, phosphorous removal to the extent required is not possible. Therefore, the addition of nitrogen fertilizers were considered. Addition of fertilizer requires that the energy of the fertilizer produced be taken into account for the overall process.

Assuming all other elements are available in excess (carbon from CO_2 in the atmosphere and water and oxygen and hydrogen from water etc.), the concentrations of the nutrients are uniformly distributed throughout the volume of wastewater, and conditions (light, temperature,

etc.) are the same, the rate of nutrient assimilation from the wastewater will be a function of the productivity of the growth system being used. This study does not incorporate the addition of carbon dioxide from external sources, such as flue gas.

Productivity of suspended systems varies widely depending on the conditions the algae are subjected to. Based on Sturm et al. [2] wastewater used for the growth of algae in suspended form for nutrient removal can range from 5 to 16 mg m^{-2} day^{-1}. An average value of 10 mg m^{-2} day^{-1} will be used for this study. The pilot scale RABR can achieve productivities of between 20 to 31 mg m^{-2} day^{-1}. For this study an average value of 25 mg m^{-2} day^{-1} will be used.

3.2. Analysis of suspended system

Based on the volume and concentration of wastewater used there is a total of 7.8 g/m3 nitrogen and 4.5 g/m3 phosphorous available for the algal cells to assimilate. To reach final target concentrations of 1.5 g/m^3 (1.5 mg/L) and 1.0 g/m^3 phosphorous, a total of 3 g/m^3 and 3.5 g/m^3 need to removed from each m^3 of wastewater respectively. Using the parameters stated the rate of nutrient uptake for each element was determined and is presented in Table B1.

Table B1. Rate of nutrient uptake by algae growing in suspended form.

Element:	**Uptake Rate: (g/day m^3)**
Carbon	15.41
Hydrogen	3.19
Oxygen	21.32
Nitrogen	2.71
Phosphorous	0.38

However, because of the mismatch in the molar ratio between nitrogen and phosphorous in the wastewater (3.8: 1) and algal biomass (16: 1), removal of 3.0 and 3.5 g/m^3 is not possible. The maximum amount of phosphorous that can be removed using the wastewater without

modification is 1.08 g/m^3 phosphorous, which corresponds to a final phosphorous concentration of 3.42 g/m^3, well of above the target concentrations of 1.5 g/m^3 or 1.0 g/m^3.

3.2.1. Nitrogen supplementation of raceway wastewater for phosphorous removal

To reach the target phosphorous concentrations stated, the wastewater will require nitrogen supplementation to balance the nutrients according to the stoichiometric needs of the algal biomass. This involves the addition of nitrogen fertilizers to the wastewater. A number of different sources of nitrogen can be used as fertilizer including sodium nitrate, ammonia, urea, and others. For this analysis the addition of urea will be used. Addition of ammonia and sodium nitrate will be discussed as comparison. The addition of ammonia leads to the potential of nitrogen loss by stripping or volatilizing, which would need to be considered if used.

The first scenario (1) will involve increasing the nitrogen concentration in the wastewater to remove enough phosphorous to reach 1.5 g/m^3 concentration. To reach this concentration an additional 21.7 g/m^3 of nitrogen needs to be added to the wastewater. The second scenario (2), to reach a final phosphorous concentration of 1.0 g/m^3, requires the addition of 25.3 g/m^3 of nitrogen.

The energy associated with the production of these fertilizers was determined based on the GREET model. From the GREET database it was found that the production of ammonia, urea, and sodium nitrate required 32.577, 21.855, and 7.681 mmBTU/ton fertilizer produced respectively. The GREET values account for the production process and well as feedstock related activities. Table B2 summarizes the amounts of nutrients added as well as the associated energy with the mass of fertilizer added.

Table B2. Masses of fertilizer required to reach target phosphorous concentrations of 1.5 and 1.0 g/m^3 and energies associated with each fertilizer's production.

Nitrogen Source: (Scenario #)	Mass fraction of nitrogen in fertilizer: (g/g)	Mass of Fertilizer added: (g)	Production Energy of Fertilizer: (KJ/g)	Energy associated with Fertilizer Addition: (KJ/m^3)
Ammonia (1)	0.87	15.95	37.6	599.72
Urea (1)	0.47	29.53	25.2	744.16
Sodium Nitrate (1)	0.16	86.75	8.9	772.01
Ammonia (2)	0.87	20.82	37.6	782.83
Urea (2)	0.47	37.21	25.2	937.69
Sodium Nitrate (2)	0.16	109.31	8.9	972.86

With the addition of nutrients it is possible to reach the target phosphorous concentrations. Based on the nutrient uptakes rates presented in Table B1, target phosphorous concentrations can be reached within 7.9 and 9.2 days for 1.5 g/m^3 and 1.0 g/m^3 per nitrogen supplemented m^3 wastewater respectively.

However, the addition of fertilizer to supplement the wastewater leads to an additional energy burden that needs to be accounted for. Without the addition of fertilizer, removal of the necessary phosphorous is not possible, other than the use of chemical or mechanical methods. Another option is to recycle the effluent from an anaerobic digester. For the Logan lagoon system an anaerobic digester will be used to digest and convert residual algal biomass to methane. Therefore, the potential exists for taking the liquid effluent from the digester and increasing the nitrogen concentration of the wastewater. This option will be discussed in future sections.

3.2. Analysis of RABR system for nutrient removal

The wastewater used for this analysis is the same as for the suspended system and because the same unit volume of 1 m^3 is used the amounts of nitrogen and phosphorous remain the same. Using the productivity of the RABR the rate of nutrient uptake for each element was determined and is presented in Table B3.

Table B3. Rate of nutrient uptake by algae growing in suspended form.

Element:	**Uptake Rate: (g/day m^3)**
Carbon	38.5
Hydrogen	8.0
Oxygen	53.3
Nitrogen	6.78
Phosphorous	0.94

Due to the characteristics of the wastewater between the analysis of the raceway and RABR being the same, nitrogen supplementation data will be the same between the two systems. Therefore, amounts of nitrogen addition will remain constant for the two systems as summarized in Table B2. The only parameters that will change will be the rate at which the RABR system will achieve the target phosphorous concentrations.

When using the RABR the concentration of phosphorous can be reduced from 4.5 g/m3 to 1.5 g/m^3 or 1.0 g/m^3 in 3.2 and 3.7 days per m^3 wastewater respectively. This is lower than the raceway, which can achieve the required concentrations in 7.9 and 9.2 days per m^3 wastewater respectively. The higher productivity of the RABR is the main factor in the quicker removal of phosphorous from the wastewater. However, an important point is that because of the higher nutrient uptake rate, the residence time of the wastewater within a RABR system is much lower than for a raceway system, thus leading to the remediation of larger volumes of wastewater in the same time. This can lower the land footprint of the system for equivalent levels of nutrient removal.

4. Energy Analysis of Raceway and the RABR Growth and Harvesting Systems

4.1. Introduction

An energy balance was performed over both growth and harvesting methods discussed.

For each system the base unit was the area of a pilot scale RABR (4.3 m^2). Therefore, the volumes of wastewater analyzed depended on the depth of the liquid for each system. Typically algal raceway ponds are approximately 30 cm (11.8 in) deep, while the RABR requires a liquid depth of 36 in. The corresponding volumes were used as the basis for this energy balance.

The energy balance accounted for all energy inputs and outputs for each system involved in the growth and harvesting of algal biomass. The functional unit for this analysis was the production of enough algal biomass to generate 1 kg of dry algal biomass. Power to pump wastewater was neglected due to the Logan lagoons facility being operated by the City of Logan Environmental Department, which has responsibility of wastewater movement.

4.2. Energy balance around the RABR System

For this study a pilot scale RABR system was analyzed. Such a system has been described in detail by Christenson et al [17]. Figure B1 illustrates the pilot scale RABR growth and harvesting system. This system is unique in that the growth and harvesting systems are combined into a single unit. As the RABR rotates in the wastewater, algal cells are able to attach and grow on the substrate. Thus, when the RABR is ready to harvest, the rope is scraped off and the harvested algal biomass can be further processed.

The RABR only requires power to rotate. The harvesting mechanism uses the same power for rotation of the RABR. Due to the harvesting process adding a small amount of friction for a short amount of time, the additional energy input to harvest is considered negligible. Power required to rotate the RABR has been calculated as 6 Watts [17]. Based on this value the energy needed to generate 1 kg of algae can be calculated as follows:

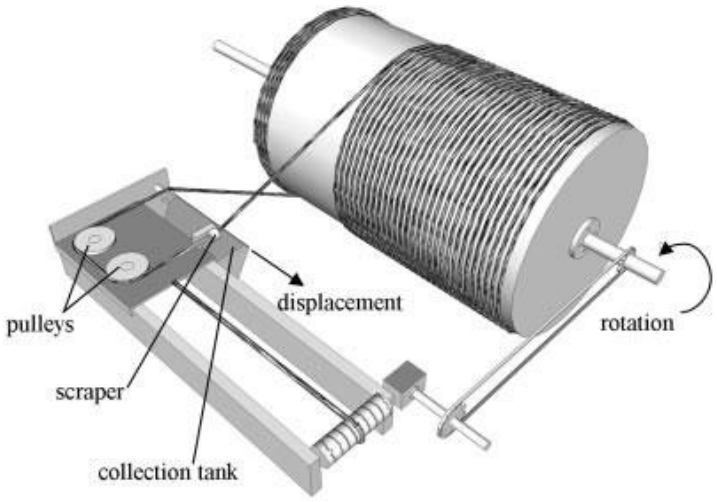

Figure B1. RABR growth and harvesting system [17].

Equation B1. $$(4.3\ m^2) * \left(25\ \frac{g}{m^2\ day}\right) = 107.5\ \frac{g}{day}$$

Equation B2. $$6\ watts = \left(6\ \frac{J}{s}\right) * \left(3{,}600\ \frac{s}{hour}\right) * \left(24\ \frac{hours}{day}\right) = 518{,}400\ \frac{J}{day}$$

Equation B3. $$\frac{518{,}400\ \frac{J}{day}}{107.5\ \frac{g}{day}} = \ 4{,}822\ \frac{J}{g} = 4{,}822\ \frac{KJ}{Kg}$$

The first analysis of the RABR energy requirements involved using wastewater without any modifications. This will serve as a baseline scenario to compare to scenarios where the wastewater has been supplemented with nitrogen, as discussed in section 3.2.1. Wastewater being fed to the RABRs are proposed to contain nitrogen and phosphorous at concentrations of 7.8 mg/L and 4.5 mg/L respectively. With these concentrations the algae have available to them 30.67 g N and 17.69 g P. Assuming no other nutrient is limiting algal biomass yield is based on the amount of nitrogen present in the wastewater. Biomass yield was calculated based on the Redfield expression for algae [16].

As stated the energy input into the RABR system is electrical energy for rotation of the system. The energy output from the RABR is the algal biomass. Energy density of algal biomass

has been estimated as 21.4 KJ/g dry algae [17]. Figure B2 illustrates energy inputs and outputs for the RABR system. Table B4 summarizes the energy inputs and output from the RABR system for the generation of 1 kg of biomass. The energy associated with fertilizer will apply for future scenarios analyzed, but not for the baseline analysis. Table B4 presents the energy inputs and outputs from the baseline RABR system.

It should be noted that although the nutrient concentrations are changing by addition of urea, the productivity does not change. This is due to the lack of data on the effect of nutrient concentration on the growth rates of algal biofilms. Therefore, in the energy balances performed for the different scenarios the amount of energy taken to grow 1 kg of algal biomass remains constant. When in reality the energy required to grow 1 kg of algal biomass at optimum nutrient concentrations may be lower (higher productivity leads to lower growth times), thus giving motivation, from an energetic perspective, to supplement with nitrogen fertilizers. Without proper data though, that aspect cannot be analyzed.

Table B4. Summary of energy inputs and output from the RABR system for generating algal biomass for 1 kg of algal biomass. This analysis is for the baseline scenario (no supplementation of the wastewater with fertilizer)

Stream:	**Energy Input: KJ/kg dry biomass**	**Energy Output: KJ/kg dry biomass**
Electrical energy for RABR rotation:	(-4822.3)	n/a
Energy associated with urea:	0	n/a
Energy associated with harvested algal biomass:	n/a	(+24,100)
Net Energy:	**(+19,277.7)**	

As discussed in section 3.2.1., the wastewater used as the growth medium needs to be supplemented with nitrogen containing fertilizer for the proper remediation of the wastewater being processed. For this analysis the energy associated with the production of the fertilizer was accounted, but only for the addition of urea. Urea is considered due to its lower energy

requirement per mass fertilizer added and also because urea does not volatilize into the atmosphere after its addition to water, as is the case for ammonia. To reach the target of 1.5 g/m^3 and 1.0 g/m^3 approximately 46.2 and 53.8 g/m^3 must be added. This corresponds to an energy equivalent of 1163.5 and 1365.5 KJ per m^3 of wastewater supplemented respectively.

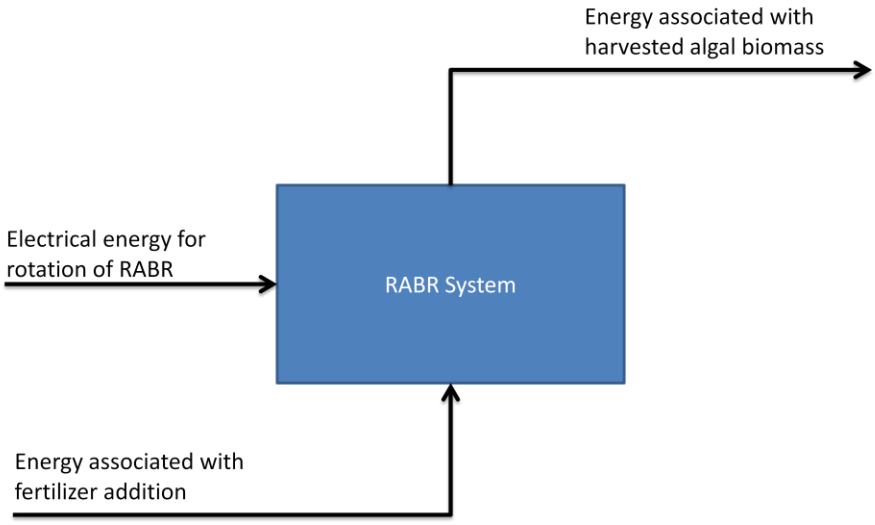

Figure B2. Energy balance around the RABR.

Table B5. Summary of energy inputs and output from the RABR system for generating algal biomass for 1 kg of algal biomass. Using a target phosphorous concentration of 1.5 g/m^3.

Stream:	**Energy Input: KJ/kg dry biomass**	**Energy Output: KJ/kg dry biomass**
Electrical energy for RABR rotation:	(-4822.3)	n/a
Energy associated with urea:	Scenario 1: (-2165.6) Scenario 2: (-2340.0)	n/a
Energy associated with harvested algal biomass:	n/a	(+24,100)
Net Energy:	**Scenario 1: (+17,112.7)** **Scenario 2: (+16,937.7)**	

4.3. Energy balance around a raceway and harvesting system

The raceway and harvesting system modeled in this study is similar to many studied by several authors [13,18–20]. This model makes use of dissolved air flotation (DAF) and centrifugation for the harvesting of algal biomass from the raceway. Major energy inputs into this system

include mixing power for the raceway pond, energy for the DAF unit, energy for centrifugation, and energy for drying the algal biomass. Total algal biomass generated from each unit area (4.3 m^2) is based on the time required to reach the desired phosphorous concentration (section 3.2.1.) and the productivity. For 1.5 g/m^3 and 1.0 g/m^3 phosphorous concentrations, the total biomass generated is 443 g and 516 g respectively. For the baseline case, no nitrogen supplementation, the mass of algae generated within the raceway unit volume was the maximum allowable based on the nitrogen available for algal growth. This resulted in the growth of 159.3 g algae.

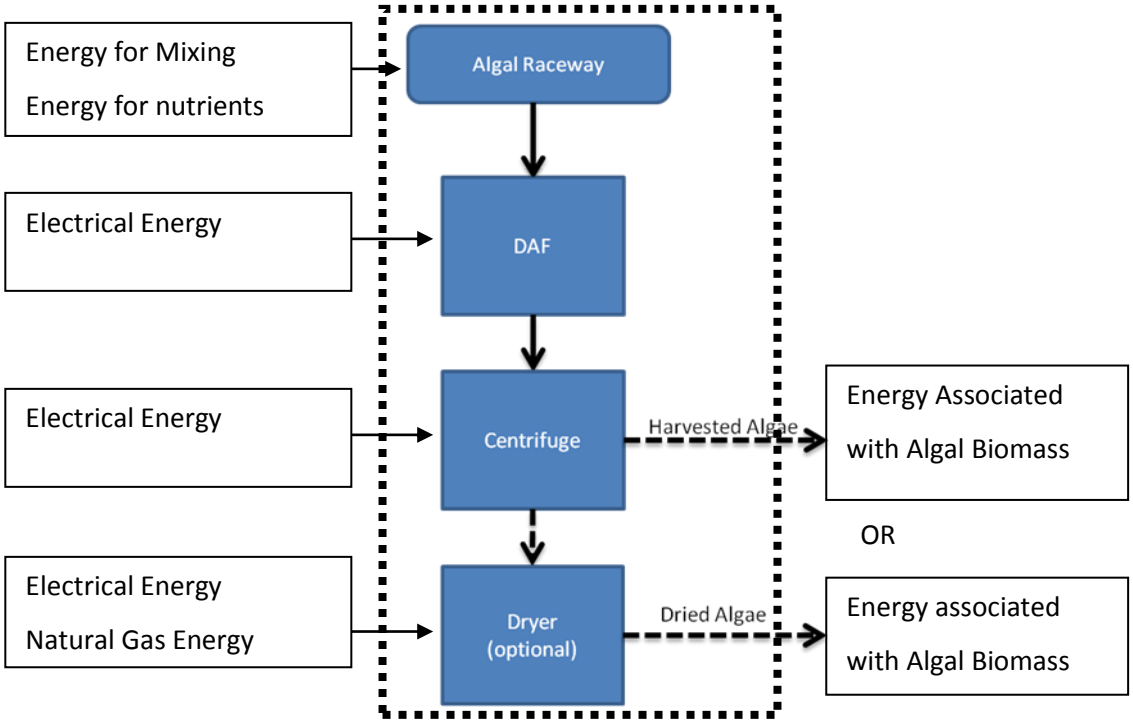

Figure B3. Illustration of growth and harvesting system for a raceway pond for algae.

Drying of the algal biomass is not required for some processes, as they are able to extract lipids from wet algal biomass [13,20], but in most cases the algal biomass is dried prior to processing [12,21] . This issue will be discussed in future sections. Table B6 presents energy consumption data for each of the major inputs and provides a literature source for each value.

Table B6. Energy consumption for major unit operations/processes.

Major process Unit/Operation:	**Energy consumption:**	**Source:**
Mixing (Paddlewheel)	0.2 W/ha	[4]
Dissolved Air Flotation:	0.5 KWh/m^3	[17]
Centrifugation:	28.8 MJ/m^3	[13]
Dryer: (natural gas)	3556 KJ/kg water removed	[22]

Energy associated with mixing is applied to an area equal to that of the RABR (4.3 m^2). With an energy requirement of 0.2 W/ha or 0.86W per RABR area and an algal productivity of 10 g m^{-2} day^{-1}, the energy required to generate 1 kg of dry algal biomass can be calculated as follows:

Equation B4: $$(4.3\ m^2) * \left(10\ \frac{g}{m^2\ day}\right) = 43\ \frac{g}{day}$$

Equation B5: $$0.86\ watts = \left(0.86\ \frac{J}{s}\right) * \left(3{,}600\ \frac{s}{hour}\right) * \left(24\ \frac{hours}{day}\right) = 74{,}304\ \frac{J}{day}$$

Equation B6: $$\frac{74{,}304\ \frac{J}{day}}{43\ \frac{g}{day}} = 1{,}728\ \frac{J}{g} = 1{,}728\ \frac{KJ}{Kg}$$

Wastewater containing the algal biomass is sent through a DAF unit and the algal biomass is concentrated to 4.3 wt% solids. Energy consumption to achieve this concentration is 0.5 KWh/m^3 liquid processed. Based on the area of water (4.3 m^2) and a depth of 30 cm, the total volume processed is 1289 L or 1.29 m^3. This volume corresponds to a total energy consumption of 5240 KJ/Kg and 4491 KJ/kg for phosphorous concentrations of 1.5 and 1.0 g/m^3 respectively. For the baseline case, energy consumed equals 14,561 KJ/Kg.

Equation B8: $\frac{\left(0.5\,\frac{KWh}{m^3}\right)=\left(1800\,\frac{KJ}{m^3}\right)*\,1.29\,m^3}{0.159\,kg} = 14{,}561\,\frac{KJ}{Kg}$ (Baseline Case)

Equation B9: $\frac{\left(0.5\,\frac{KWh}{m^3}\right)=\left(1800\,\frac{KJ}{m^3}\right)*\,1.29\,m^3}{0.443\,kg} = 5239\,\frac{KJ}{Kg}$ (Target: 1.5 mg/L P)

Equation B10: $\frac{\left(0.5\,\frac{KWh}{m^3}\right)=\left(1800\,\frac{KJ}{m^3}\right)*\,1.29\,m^3}{0.517\,kg} = 4491\,\frac{KJ}{Kg}$ (Target 1.0 mg/L P)

The difference in energy consumption for the two scenarios is due to the varying amount of algal biomass generated. With the baseline case the DAF is processing a suspension containing 0.012% solids. Therefore, production of an equivalent amount of algal biomass requires the processing of higher volumes of biomass. As the concentration of algal biomass increases, 0.034 and 0.04% for target phosphorous values of 1.5 and 1.0 mg/L, the energy decreases for DAF operation per kg of algal biomass generated.

The concentrated algal paste is then sent to a centrifuge to dewater further, up to a concentration of 20 wt% solids. A typical centrifuge consumes approximately 28.8 MJ/m^3 (28.8 KJ/L) of material processed [13]. Sludge generated by the DAF is 4.3 wt% solids. Sludge volumes for each scenario and energy for centrifugation are provided in Table B7. On a per kg dry algal biomass basis, the energy required is 669.8 KJ/kg and it is for all scenarios analyzed. The energy consumption does not vary per kg of algal biomass generated because the DAF is feeding the centrifuge a stream containing the same amount of solids (20%).

Table B7. Energies associated with centrifugation step for suspended algae.

Scenario:	Volume of DAF Sludge: L	Energy for Centrifuge: KJ	Energy for Centrifuge: KJ/Kg
Baseline:	3.71	106.7	669.8
Scenario 1:	10.30	296.6	669.8
Scenario 2:	12.01	346.0	669.8

Processes exist that utilize algal biomass at 20% solids and extract lipids for biofuels production [13]. If the algal biomass is to be dried it must be dried in one of multiple types of dryers available [12,18,21]. For this study it is assumed a natural gas dryer [21] is used that consumes 3556 KJ/kg algae produced [22]. Table B9 provides a summary of the energy for the dewatering process, from the raceway to the dried algal biomass.

Table B8. Energy associated with the dewatering of algal biomass from suspended cultures. For each scenario based on a unit volume of 1288.8 L.

Stream:	Energy Required KJ:	Mass of water removed: Kg
For DAF:	Baseline: 2,320 Scenario 1: 2,320 Scenario 2: 2,320	Baseline: 1,285.1 Scenario 1: 1,278.5 Scenario 2: 1,276.8
For Centrifuge:	Baseline: 106.7 Scenario 1: 296.6 Scenario 2: 346.0	Baseline: 2.64 Scenario 1: 7.35 Scenario 2: 8.57
For Dryer:	Baseline: 2,203.2 Scenario 1: 8,747 Scenario 2: 10,205	Baseline: 0.89 Scenario 1: 2.46 Scenario 2: 2.87

Table B9. Energy requirements in terms of KJ/Kg dry algae produced. Based on values from the energy values in Table B7 divided by the mass of algae generated for each scenario.

Stream:	Energy Required KJ/Kg:
Algae Generated:	Baseline: 159.3 g Scenario 1: 442.8 Scenario 2: 516.6
For DAF:	Baseline: 14,561 Scenario 1: 5,239 Scenario 2: 4,491
For Centrifuge:	Baseline: 669.8 Scenario 1: 669.8 Scenario 2: 669.8
For Dryer:	Baseline: 19,756 Scenario 1: 19,756 Scenario 2: 19,756

When drying of the algal biomass is not required the raceway growth and harvesting method can be energetically favorable, but when drying is required the energy consumed is higher than the energy gained from the algal biomass. The RABR system had a net energy of +17,113 KJ/Kg of algal biomass produced (Table B4). For the raceway system the net energy without thermal drying of the biomass was +12,804 KJ/Kg algae biomass produced. When thermal drying was required the net value was -1,024 KJ/Kg algae biomass. In either case the RABR system is energetically favorable compared to the raceway growth and harvesting system.

4.4. Summary for RABR

For the RABR the energy balance and associated CO_2 emissions will be based on Figure B2. The first case described will be for the baseline where no nutrients are added and the energy and CO_2 balance is for one RABR unit.

4.3. Summary of Energy Usage for Each Harvesting Method and CO_2 emissions

Table B10. Raw Data used for calculations:

Data For:	Value or Ratio Used:	Source/Comments:
Electrical Energy Generation	30% Natural Gas 70% Coal Fired	[23]
Electrical Energy CO2 Emissions	Coal: 1,179 g CO2/KWh Natural Gas: 549 g CO2/KWh	GREET (Electricity at Wall Outlets)
Energy associated with Algae:	21.4 KJ/g	Christenson et al.
Energy Associated with Urea:	21.855 mmBTU/ton Urea	GREET
CO2 Associated with Urea:	691,732 g CO2/ton Urea	GREET
Natural Gas Dryer Emissions:	59,379 g CO2/mmBTU	GREET Utility/Industrial Boiler

Table B11. For Baseline RABR Unit:

Stream:	**Energy Input: KJ/kg**	**Energy Output: KJ/kg**	**g CO2/kg biomass Input/Output:**
Electrical energy for RABR rotation:	(-4,822)	-	(+1,326) Emissions
Energy associated with harvested algal biomass:	-	(+21,400)	(-1,313) Taken by Algae
Net:	**(+16,578)**		**(+13)**

Table B12. For the RABR Scenario 1: (1.5 mg/L P)

Stream:	**Energy Input: KJ/kg**	**Energy Output: KJ/kg**	**g CO2/kg biomass Input/Output:**
Electrical energy for RABR rotation:	(-4822)	-	(+1,326) Emissions
Energy Associated with Urea Addition:	(-2,165)	-	(+65) Emissions
Energy associated with harvested algal biomass:	-	(+21,400)	(-1,313) Taken by Algae
Net:	**(+14,412)**		**(+78)**

Table B13. For the RABR Scenario 2: (1.0 mg/L P)

Stream:	**Energy Input: KJ/kg**	**Energy Output: KJ/kg**	**g CO2/kg biomass Input/Output:**
Electrical energy for RABR rotation:	(-4,822)	-	(+1,326) Emissions
Energy Associated with Urea Addition:	(-2,340)	-	(+71) Emissions
Energy associated with harvested algal biomass:	-	(+21,400)	(-1,313) Taken by Algae
Net:	**(+14,238)**		**(+84)**

4.5. Summary for Suspended Algae

For the raceway the energy balance and associated CO_2 emissions will be based on Figure B3. The first case described will be for the baseline where no nutrients are added and the energy and CO_2 balance is for one RABR unit. Natural gas dryer used is based on an industrial/utility reboiler and does not use electricity.

Table B14. For Baseline Raceway Unit:

Stream:	**Energy Input: KJ/kg**	**Energy Output: KJ/kg**	**g CO2/kg biomass Input/Output:**
Electrical energy for mixing:	(-1,728)	-	(+475) Emissions
Electrical energy for DAF:	(-14,561)	-	(+4,004) Emissions
Electrical energy for Centrifuge:	(-670)	-	(+184) Emissions
Energy associated with harvested algal biomass:	-	(+21,400)	(-1,313) Taken by Algae
NET	**(+4,441)**		**(+3,350)**
Energy associated with drying:	(-19,756)	-	(+1,126) Emissions
Net:	**(-15,315)**		**(+4476)**

Table B15. For Scenario 1 Raceway Unit:

Stream:	**Energy Input: KJ/kg**	**Energy Output: KJ/kg**	**g CO2/kg biomass Input/Output:**
Electrical energy for mixing:	(-1,728)	-	(+475) Emissions
Energy for Urea Production:	(-2,165)	-	(+65)
Electrical energy for DAF:	(-5,239)	-	(+1441) Emissions
Electrical energy for Centrifuge:	(-670)	-	(+184) Emissions
Energy associated with harvested algal biomass:	-	(+21,400)	(-1,313) Taken by Algae
NET	**(+11,598)**		**(+852)**
Energy associated with drying:	(-19,756)	-	(+1,126) Emissions
Net:	**(-8,158)**		**(+1,860)**

Table B16. For Scenario 2 Raceway Unit:

Stream:	**Energy Input: KJ/kg**	**Energy Output: KJ/kg**	**g CO2/kg biomass Input/Output:**
Electrical energy for mixing:	(-1,728)	-	(+475) Emissions
Energy for Urea Production:	(-2,340)	-	(+71)
Electrical energy for DAF:	(-4,491)	-	(+1,235) Emissions
Electrical energy for Centrifuge:	(-670)	-	(+184) Emissions
Energy associated with harvested algal biomass:	-	(+21,400)	(-1,313) Taken by Algae
NET	**(+12,171)**		**(+652)**
Energy associated with drying:	(-19,756)	-	(+1,008) Emissions
Net:	**(-7,585)**		**(+1,660)**

4.6. Mass Balance Analysis

An overall mass balance was performed from the influent wastewater to bio-product production. This was performed using a basis of generating 1 Kg of algal oil. Figure B4 illustrates the generic mass balance for the entire process described.

The mass balance was performed for all three scenarios, with each one using the same functional unit of 1 Kg algal oil. Table B18 presents the data generated. Table B19 presents data for the baseline raceway scenario.

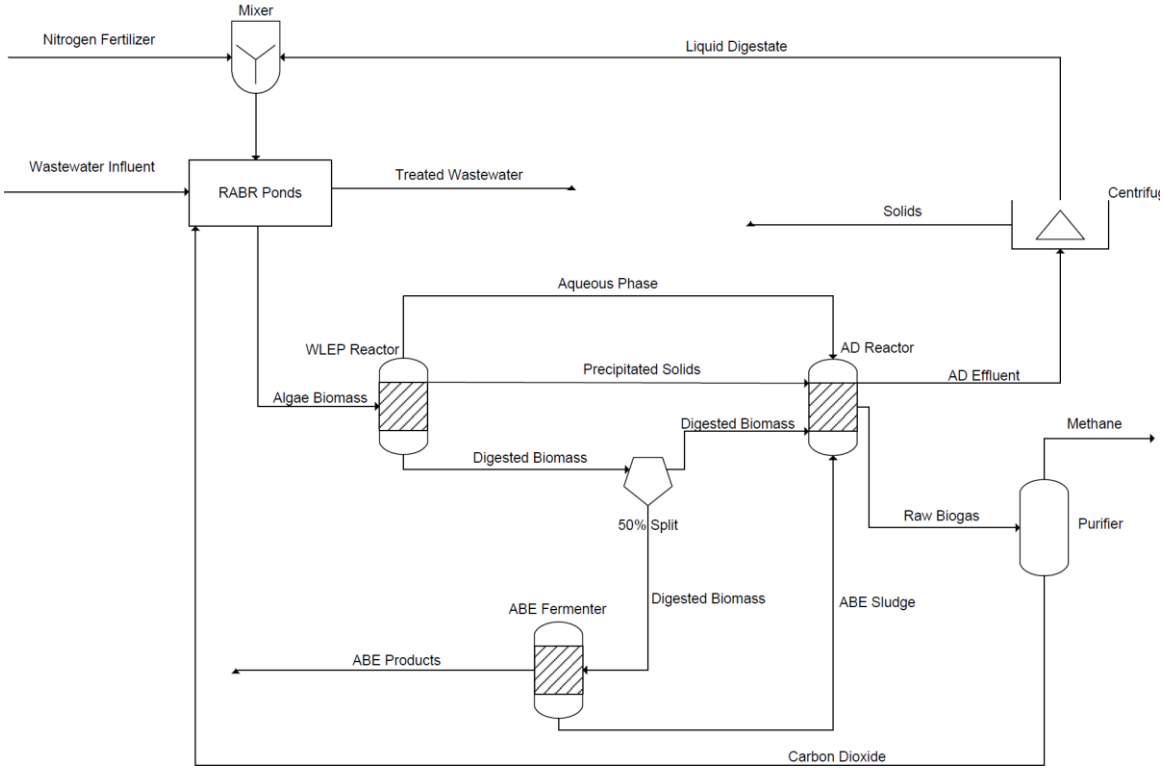

Figure B4. Flow diagram describing the growth of algal biomass to the production of bioproducts.

Table B17. Mass balance results for three scenarios of phosphorous removal. RABR productivity is assumed to be 25 g/m^2 day and each unit is 4.3 m^2.

Stream/Parameter:	**Units:**	**Baseline:**	**Scenario 1:**	**Scenario 2:**
Influent Wastewater:	m3/day	80.68	15.48	13.19
Number of RABRs Required:	Units	93.02	93.02	93.02
Total RABR Area Required:	m2	400.00	400.00	400.00
Supplemental N:	g/day	0.00	57.32	75.18
Liquid Digestate:	m3/day	0.00	0.60	0.60
Algal Oil Collected:	Kg/day	1.00	1.00	1.00
ABE Products:	Kg/day	0.44	0.44	0.44
Carbon Dioxide:	Kg/day	0.00	2.13	2.13
Methane Gas:	Kg/day	0.00	1.65	1.65

Table B18. Baseline raceway scenario for mass balance assuming 10 g/m^2 day. Area required to grow the algal biomass increases due to the decrease in productivity.

Stream/Parameter:	**Units:**	**Baseline:**
Influent Wastewater:	m3/day	80.68
Total Raceway Area Required:	m2	1000.00
Algal Oil Collected:	Kg/day	1.00
ABE Products:	Kg/day	0.44

Table B19. Area requirements for RABR assuming 31 g/m^2 day. Area remains constant for all scenarios.

Stream/Parameter:	**Units:**	**Baseline:**
Influent Wastewater:	m3/day	80.68
Number of RABRs Required:	Units	93.02
Total RABR Area Required:	**m2**	**322**
Supplemental N:	g/day	0.00
Liquid Digestate:	m3/day	0.00
Algal Oil Collected:	Kg/day	1.00
ABE Products:	Kg/day	0.44
Carbon Dioxide:	Kg/day	0.00
Methane Gas:	Kg/day	0.00

The baseline scenario assumes no anaerobic digester and no supplemental nitrogen. With this scenario the effluent phosphorous is assumed to be equal to 3.42 g/m^3. This is the effluent resulting from the removal of the corresponding amount of nitrogen by the algal biomass. Because the lagoons influent (7.8 g/m^3 N and 4.5 g/m^3 P) is nitrogen limited a large volume of wastewater is required to achieve the 10,000 Kg of dry algal biomass to generate 1 Kg algal oil. Once the anaerobic digester comes online and additional nitrogen is supplied via an external source, the wastewater demand reduces to 15.48 and 13.19 m^3/day for scenario 1 and 2 respectively. However, with increasing phosphorous removal, the amount of external nitrogen required is higher going from 57 to 75 g/day added for scenario 1 and 2 respectively.

4.7. Overall Energy Balance and CO_2 Emissions for System

For this section the energy requirements and the generated CO_2 will be determined. For comparison data from Frank et al. is presented in Table B21 [20]. Following Table B10, a series of tables are presented containing similar data generated using the RABR and the WLEP methods. For all cases electricity and heat generation are based on energy produced from the combined heat and power (CHP) unit, which is fed methane from the anaerobic digester. The data in Tables 22 through 25 assume the Redfield stoichiometric ratio for algal biomass[16], while Frank et al use 103 : 11: 1 (C: N: P). It is also assumed that the change in lipid concentration in the algae does not affect the amount of biogas generated and the composition of the solid digestate from the anaerobic reactor.

Table B20. Energy data as calculated by Frank et al. Originally presented as BTU/kg algal oil. This data is based on an algal lipid concentration of 25% by dry mass.

	KWh/kg-algal oil
Total On-Site Electricity Generation:	**3.87**
On-Site Electricity Demand:	**5.15**
Net Electrical Energy:	**-1.28**
	KWh/kg-algal oil
Total On-Site Heat Generation:	**5.00**
On-Site Heat Requirement:	**3.44**
Net Heat Energy:	**1.56**
	kg/kg algal-oil
AD Residue or Fertilizer:	**2.6**
	kg/kg algal-oil
Total CO2 Emissions:	**3.47**
Recovered CO2 used for Algae Growth:	**3.47**

Table B21. Data generated using RABR and WLEP mass and energy balances.

USING WLEP PROCESS WITH RABR: (10% Lipid Content & 25 g/m2 day)	
	KWh/kg-algal oil
Total On-Site Electricity Generation:	3.68
On-Site Electricity Demand:	14.32
Net Electrical Energy:	**-10.65**
	KWh/kg-algal oil
Total On-Site Heat Generation:	7.46
On-Site Heat Requirement:	24.41
Net Heat Energy:	**-16.95**
	kg/kg-algal oil
AD Residue or Fertilizer:	**5.73**
	kg/kg algal-oil
Total CO2 Emissions:	21.32
Recovered CO2 used for Algae Growth:	**2.13**

Table B22. Data generated using RABR and WLEP mass and energy balances.

USING WLEP PROCESS WITH RABR: (25% Lipid Content & 25 g/m2 day)	
	KWh/kg-algal oil
Total On-Site Electricity Generation:	3.68
On-Site Electricity Demand:	13.76
Net Electrical Energy:	**-10.08**
	KWh/kg-algal oil
Total On-Site Heat Generation:	7.46
On-Site Heat Requirement:	10.28
Net Heat Energy:	**-2.82**
	kg/kg-algal oil
AD Residue or Fertilizer:	**5.73**
	kg/kg algal-oil
Total CO2 Emissions:	17.86
Recovered CO2 used for Algae Growth:	**2.13**

Table B23. Data generated using RABR and WLEP mass and energy balances.

USING WLEP PROCESS WITH RABR: (10% Lipid Content & 31 g/m2 day)	
	KWh/kg-algal oil
Total On-Site Electricity Generation:	3.68
On-Site Electricity Demand:	11.73
Net Electrical Energy:	**-8.05**
	KWh/kg-algal oil
Total On-Site Heat Generation:	7.46
On-Site Heat Requirement:	19.53
Net Heat Energy:	**-12.07**
	kg/kg-algal oil
AD Residue or Fertilizer:	**5.73**
	kg/kg algal-oil
Total CO2 Emissions:	18.75
Recovered CO2 used for Algae Growth:	**2.13**

Table B24. Data generated using RABR and WLEP mass and energy balances.

USING WLEP PROCESS WITH RABR: (25% Lipid Content & 31 g/m2 day)	
	KWh/kg-algal oil
Total On-Site Electricity Generation:	3.68
On-Site Electricity Demand:	11.17
Net Electrical Energy:	**-7.49**
	KWh/kg-algal oil
Total On-Site Heat Generation:	7.46
On-Site Heat Requirement:	10.28
Net Heat Energy:	**-2.82**
	kg/kg-algal oil
AD Residue or Fertilizer:	**5.73**
	kg/kg algal-oil
Total CO2 Emissions:	15.30
Recovered CO2 used for Algae Growth:	**2.13**

Data presented in Tables B11 through B14 shows that the RABR and WLEP methods combined, demand higher heat and electrical energy than the CHP unit can provide. Research by Frank et al. found that enough heat can be supplied. However, Frank et al. requires that electrical energy be imported to the site to run the required processes.

One explanation for the high heat demand is the WLEP process. The acid and base hydrolysis steps require a significant amount of heat as shown in Table B26. Because this procedure has been recently developed, the steps have not optimized, and therefore, energy requirements are in excess. There is potential to reduce the energy demand for this step, thus lowering the overall heat requirements for the WLEP process. However, this requires further research and is outside the scope of this proposal/project.

Table B25. Energy demands for the WLEP.

WLEP Energy For Oil:	Heat: KWh	Electricity: KWh
Hydrolysis Step:	16.84	0.27
Precipitate Collection:	0.00	0.62
Lipid Extraction:	1.14	0.033
Solvent Recovery:	1.55	0.00
TOTALS:	19.53	0.93
After Efficiency Factors:	24.41	0.93

The electrical demand originates from the RABR system mainly. This value can be found in Section 4.5. Converting the 4822 KJ/kg algae to KWh/kg oil, results in an energy consumption of 13.39 KWh/kg algal oil, assuming the algal biomass is 10% lipids by dry mass.

If drying the algal biomass is required, as some lipid extraction processes call for, an additional 5.49 KWh/kg algae (10 kg dry algae for 1 kg oil) is required assuming the algae is 85% moisture (after centrifugation). This corresponds CO_2 emissions of approximately 1,100 g CO2/kg dry algal biomass (heat for drying originates from a natural gas boiler Table B6 Section 4.3)

4.8. Energy and CO_2 emissions saved or energy offset from use of wastewater

In many instances algal biomass is grown using fertilizer as the sole source of nutrients, such as nitrogen and phosphorous. However, due to the high energy demand in producing fertilizers, this becomes energetically costly. The use of wastewater has been suggested for reducing this energy demand by multiple authors [2,11]. Analysis in this section will be focused on the energy saved by the use nutrients available in the wastewater.

The Logan lagoons receives on average 15 MGD wastewater with influent concentrations of nitrogen and phosphorous at 16 mg/L and 5 mg/L respectively. However, due to nitrogen stripping and some loss of phosphorous, the concentration of nutrients being fed to the RABR

system is 7.8 mg/L nitrogen and 4.5 mg/L phosphorous. This corresponds to 7.8 g nitrogen and 4.5 g phosphorous per m^3 wastewater. Table B2 presents data on the energy required for the production of three nitrogen containing fertilizers. Table B27 summarizes the energy and CO_2 emissions saved by using wastewater and recovering nutrients from the anaerobic digester.

Table B26. Energy and CO2 credits for using wastewater and recovering nutrients via the anaerobic digester.

For the production of 1 Kg biodiesel: (10 Kg Algal Biomass)					
	Mass: (g)	**Equivalent Mass of Fertilizer: (g)**	**Fertilizer:**	**Energy to Produce Fertilizer: KJ**	**CO2 Emissions from Production:**
Nitrogen from Wastewater:	120.75	256.91	Urea	6,474.17	195.49
Phosphorous from Wastewater:	69.66	391.74	K2HPO4	10,236.07	756.05
Nitrogen Recovered from AD:	452.93	963.68	Urea	24,284.62	733.27
Phosphorous Recovered from AD:	41.70	234.48	K2HPO4	6,126.86	452.54

4.9 Overall Mass and Energy Balances:

Based on the data presented a "preliminary" mass and energy balance is presented in this section. Figure B5 presents a summarized mass balance for Scenario 1 from Table B18.

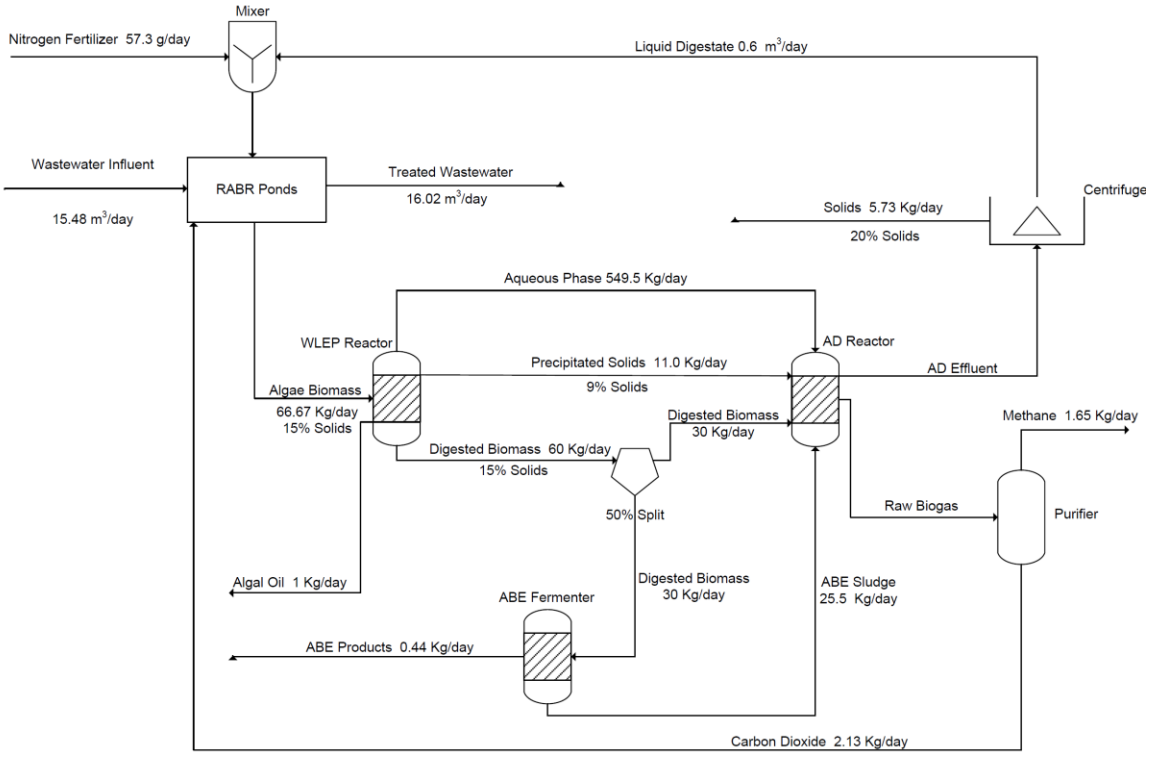

Figure B5. Illustration of a mass balance performed on the whole process. This example is based on Scenario 1 from Table B18.

Table B27. Mass flow through the process illustrated in Figure B5. (Similar to Table B9a for Scenario 1).

Stream/Parameter:	**Units:**	**Scenario 1:**
Influent Wastewater:	m3/day	15.48
Number of RABRs Required:	Units	93.02
Total RABR Area Required:	m2	400.00
Supplemental N: (Fertilizer/outside source)	g/day	57.32
Liquid Digestate:	m3/day	0.60
[Liquid Digestate] Mass of Nitrogen:	g/day	453.1
[Liquid Digestate] Mass of Phosphorous:	g/day	41.7
Algal Oil Collected:	Kg/day	1.00
ABE Products:	Kg/day	0.44
Carbon Dioxide:	Kg/day	2.13
Methane Gas:	Kg/day	1.65

An overall energy balance was also performed over the entire system. A few things should be noted: (1) Energy resulting from fuels generated are based on their combustion energies or energy densities (2) ABE fermentation energy requirements are based on a Life Cycle Analysis of Corn ABE fermentation (Wu et al. Nov 2007 "Life Cycle Assessment of Corn-Based Butanol as a Potential Transportation Fuel") (3) The energy balance is based on the above mass balance (Scenario 1). Figure B6 shows an overview of the entire energy balance, while Figure B7 illustrates the combined process.

Based on the net energies stated in Figure B6, the harvesting process generates a net energy of +162,703 KJ/day for the production of 10 kg of dry algal biomass (1 kg biodiesel). This value is similar to values presented in section 4.4. This large net energy is due to the production of algal biomass containing 21.4 KJ/g dry algae [17]. Conversion of algal biomass to fuels generates a net energy of +13,317 KJ/day based on the energy required to process the algal biomass. All energies associated with the fuels generated are based on their heats of combustion. Energy to generate biodiesel from algal oil was based on Sheehan et al.'s calculations for the transesterification of soybean biodiesel (only heat energy for transesterification and FAME purification were taken into account) [22].

However, as previously discussed in section 4.7, the CHP unit does not provide enough energy to run both the WLEP and fuel producing processes. Based on Figure B6, there is a shortage of approximately 10 KWh (38,900 KJ/day). Table B11 indicates an energy shortage of approximately 27.6 KWh. This difference may be explained due to Figure B6 not taking into account losses in energy due to inefficient conversion of the energy in the biogas to electricity and heat. Typical CHP units are 76% efficient in converting biogas derived methane to usable electricity and heat. In addition other inefficiencies have not been taken into account in Figure B6. Therefore, Figure B6 is a theoretical energy balance and further work is required to refine the energy balance.

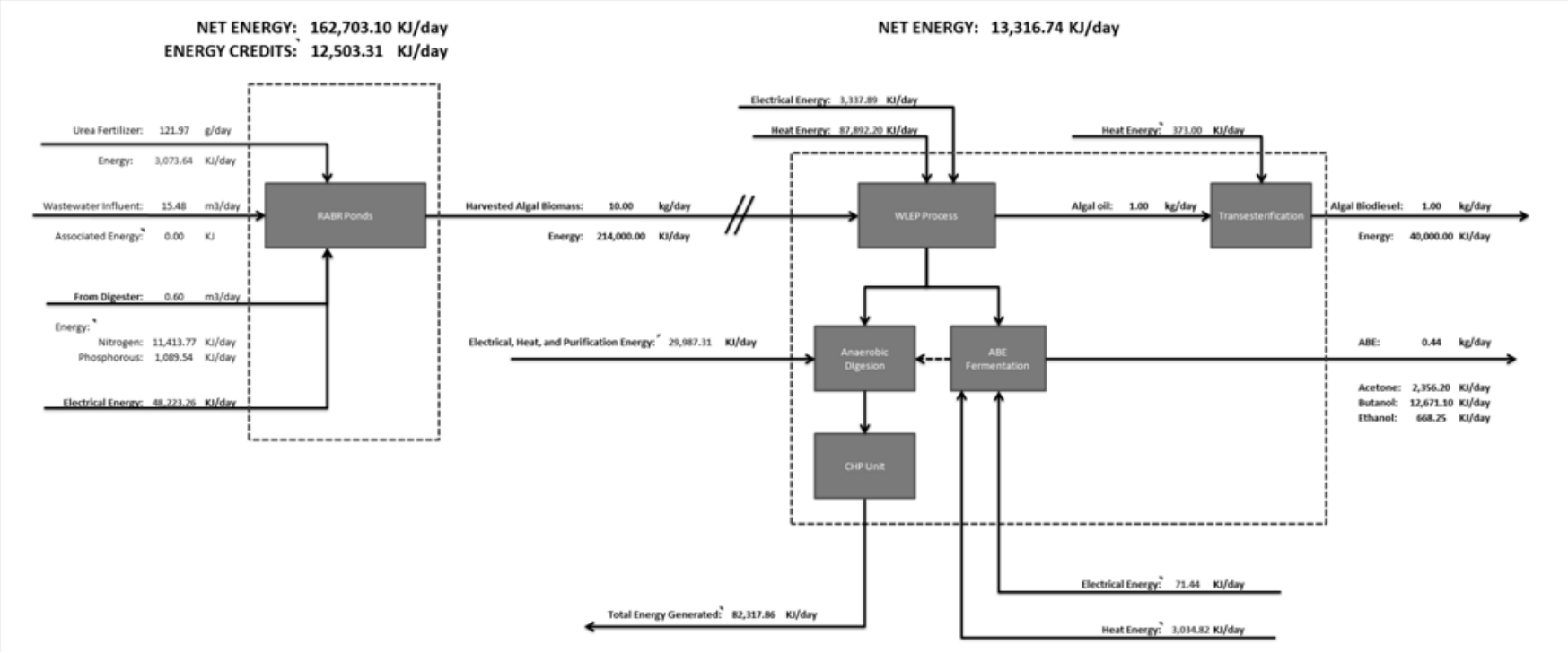

Figure B6. Energy balance for RABR harvesting and WLEP based lipid extraction process. Values indicated in red are energy streams entering the process. Energy streams in black is energy gained from the process (fuels).

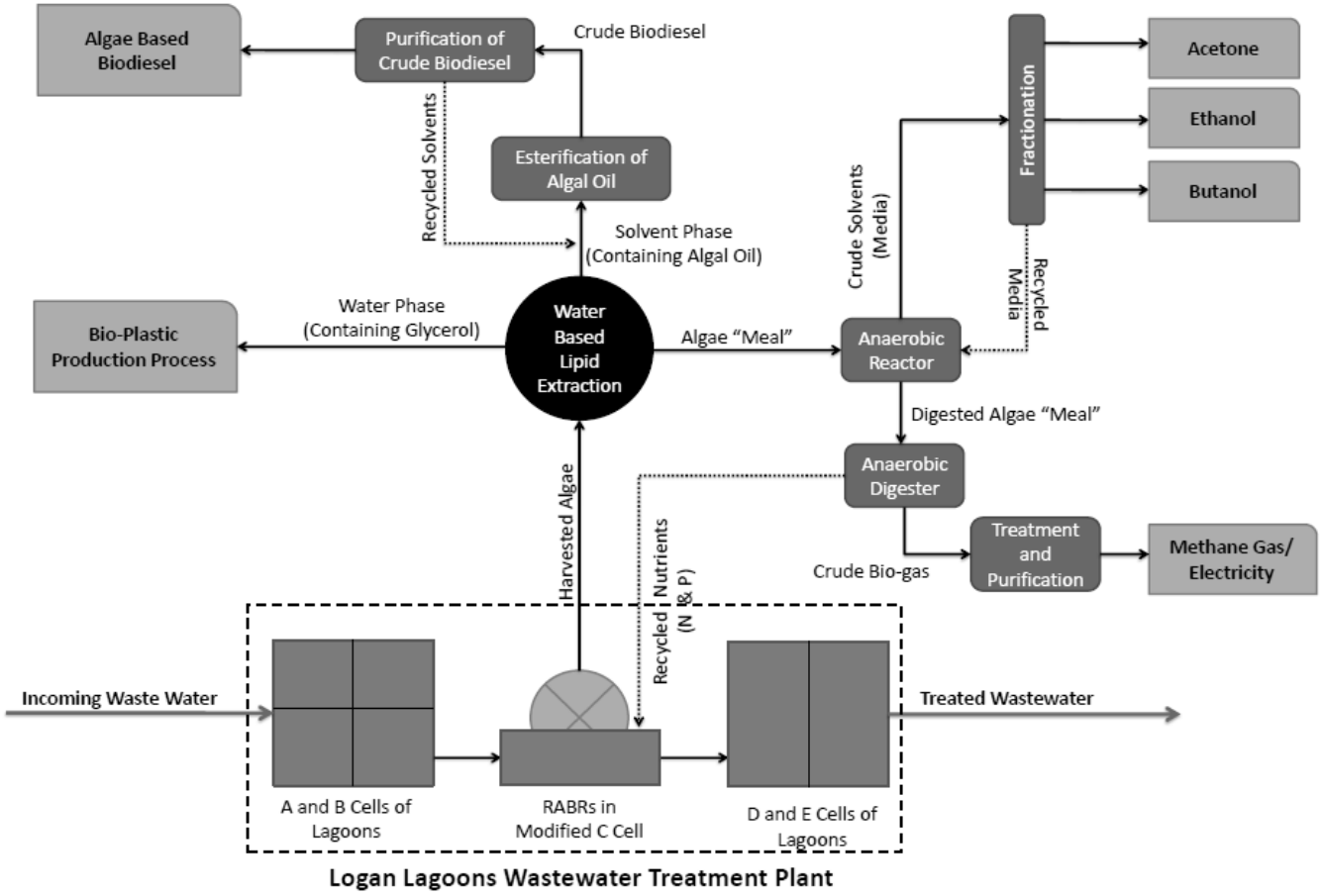

Figure B7. An illustration depicting the coupling of wastewater remediation to algal biomass processing to generate bioproducts and biofuels. Only the liquid and gas biofuels are considered for the energy balance.

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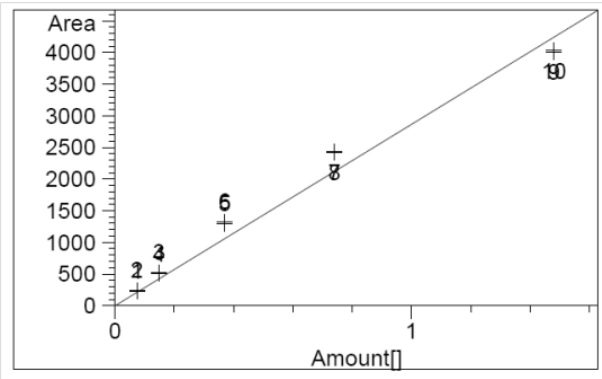
APPENDIX C

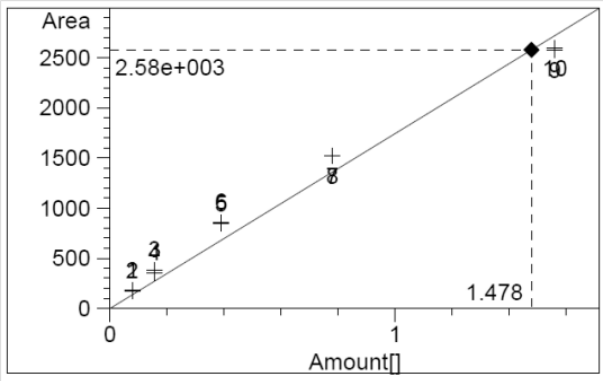
RAW DATA FOR SELECTED CHAPTERS

Gas chromatography calibration information: (Applicable for Chapters 3 and 4)



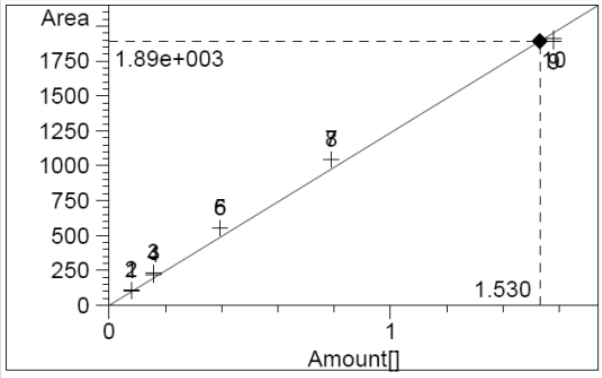
C 8:0 at exp. RT: 3.788
FID1 A, Front Signal
Correlation: 0.99556
Residual Std. Dev.: 217.65303
Formula: y = mx
m: 2867.87222
x: Amount
y: Area

Figure C1. Calibration data for C 8:0 FAME



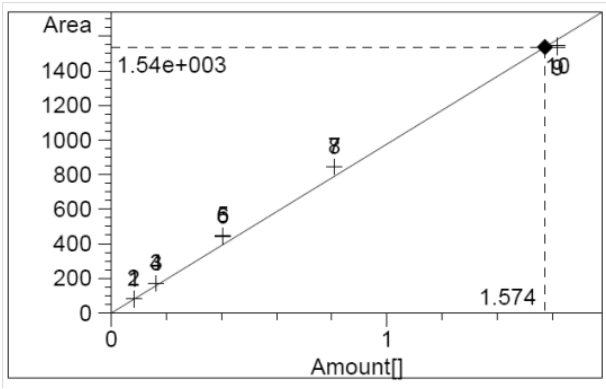
C 10:0 at exp. RT: 5.568
FID1 A, Front Signal
Correlation: 0.99576
Residual Std. Dev.: 136.54063
Formula: y = mx
m: 1745.28035
x: Amount
y: Area

Figure C2. Calibration data for C 10:0 FAME



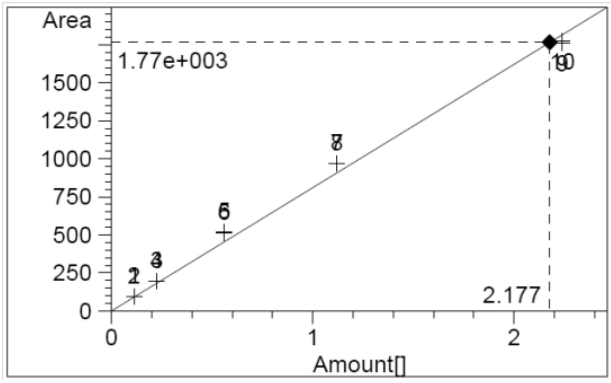
C 12:0 at exp. RT: 7.613
FID1 A, Front Signal
Correlation: 0.99872
Residual Std. Dev.: 53.62346
Formula: y = mx
m: 1237.04431
x: Amount
y: Area

Figure C3. Calibration data for C 12:0 FAME



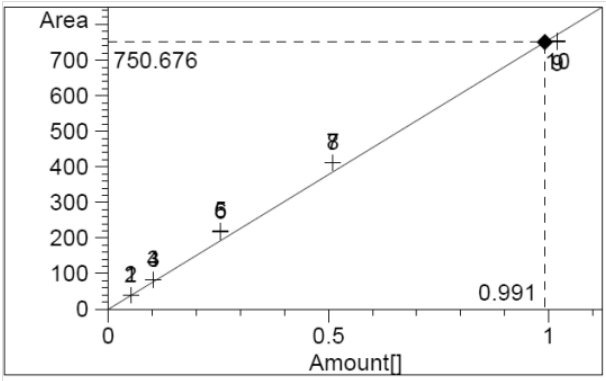
C 14:0 at exp. RT: 9.635
FID1 A, Front Signal
Correlation: 0.99892
Residual Std. Dev.: 39.89653
Formula: y = mx
m: 977.08897
x: Amount
y: Area

Figure C4. Calibration data for C 14:0 FAME



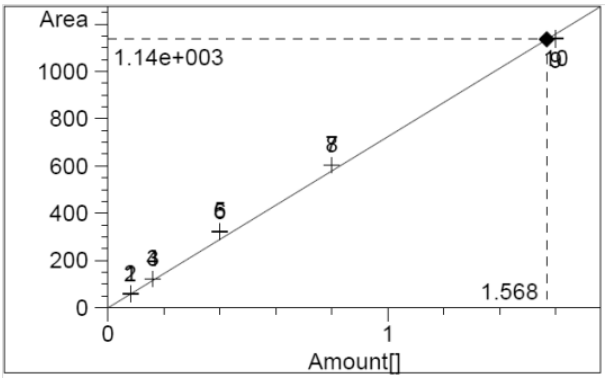
C 16:0 at exp. RT: 11.557
FID1 A, Front Signal
Correlation: 0.99888
Residual Std. Dev.: 46.62730
Formula: y = mx
m: 810.82813
x: Amount
y: Area

Figure C5. Calibration data for C 16:0 FAME



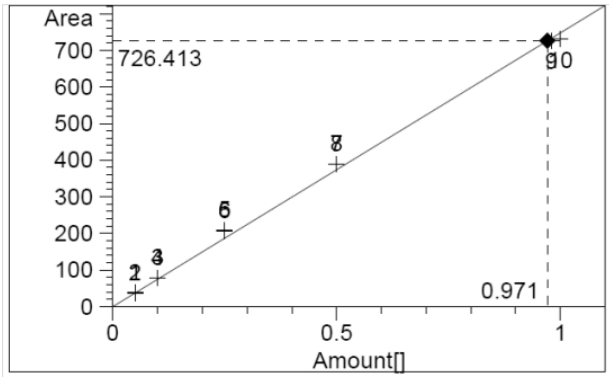
C 16:1 at exp. RT: 11.783
FID1 A, Front Signal
Correlation: 0.99888
Residual Std. Dev.: 19.86248
Formula: y = mx
m: 757.26930
x: Amount
y: Area

Figure C6. Calibration data for C 16:1 FAME



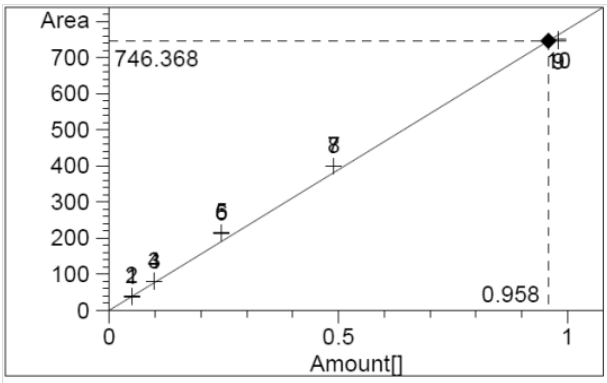
C 18:0 at exp. RT: 13.325
FID1 A, Front Signal
Correlation: 0.99942
Residual Std. Dev.: 21.38194
Formula: y = mx
m: 724.21071
x: Amount
y: Area

Figure C7. Calibration data for C 18:0 FAME



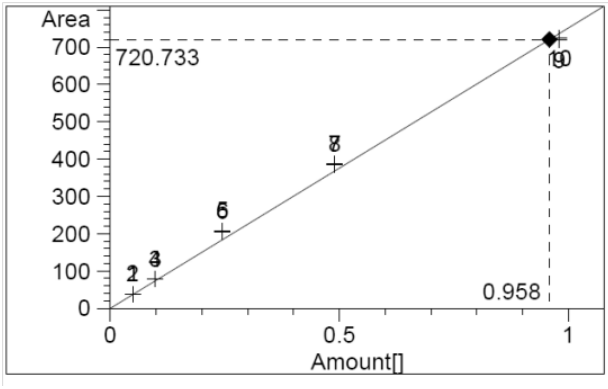
C 18:1 at exp. RT: 13.494
FID1 A, Front Signal
Correlation: 0.99946
Residual Std. Dev.: 13.27298
Formula: y = mx
m: 748.05977
x: Amount
y: Area

Figure C8. Calibration data for C 18:1FAME



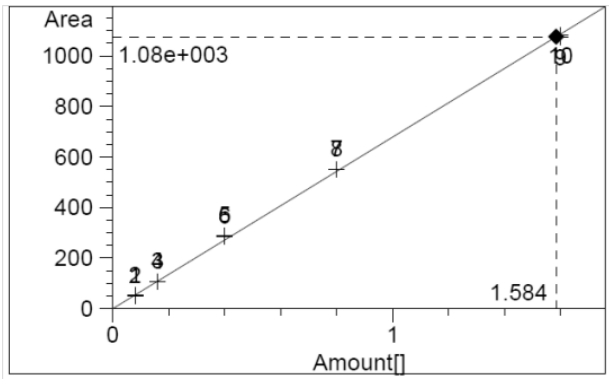
C 18:2 at exp. RT: 13.889
FID1 A, Front Signal
Correlation: 0.99929
Residual Std. Dev.: 15.65231
Formula: y = mx
m: 779.08437
x: Amount
y: Area

Figure C9. Calibration data for C 18:2 FAME



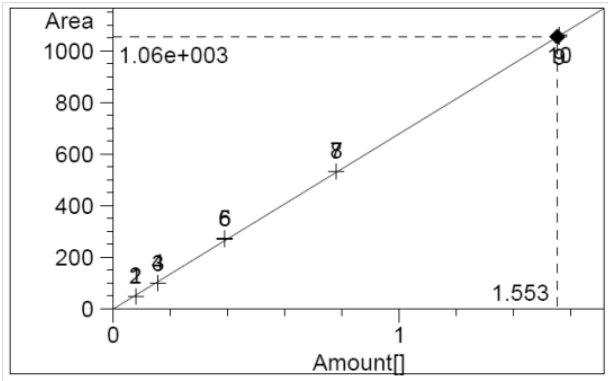
C 18:3 at exp. RT: 14.430
FID1 A, Front Signal
Correlation: 0.99930
Residual Std. Dev.: 14.98511
Formula: y = mx
m: 752.12941
x: Amount
y: Area

Figure C10. Calibration data for C 18:3 FAME



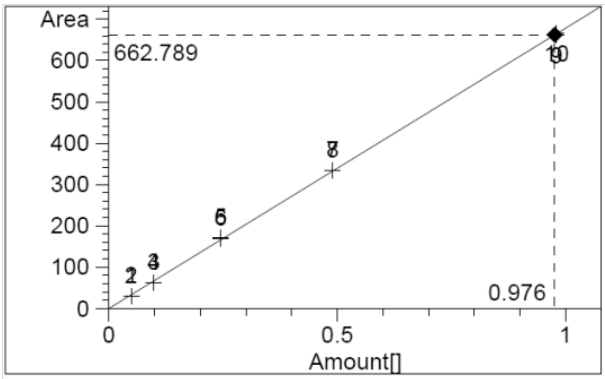
C 20:0 at exp. RT: 14.996
FID1 A, Front Signal
Correlation: 0.99987
Residual Std. Dev.: 9.58835
Formula: y = mx
m: 679.15130
x: Amount
y: Area

Figure C11. Calibration data for C 20:0 FAME



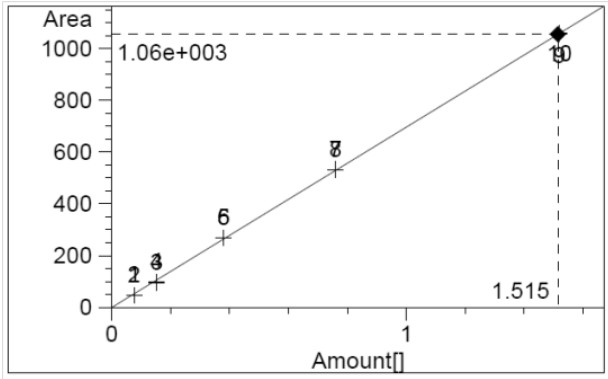
C 22:0 at exp. RT: 17.119
FID1 A, Front Signal
Correlation: 0.99995
Residual Std. Dev.: 5.80507
Formula: y = mx
m: 679.70113
x: Amount
y: Area

Figure C12. Calibration data for C 22:0 FAME



C 22:1 at exp. RT: 17.393
FID1 A, Front Signal
Correlation: 0.99996
Residual Std. Dev.: 3.38003
Formula: y = mx
m: 678.97908
x: Amount
y: Area

Figure C13. Calibration data for C 22:1 FAME



C 24:0 at exp. RT: 20.289
FID1 A, Front Signal
Correlation: 0.99995
Residual Std. Dev.: 5.90514
Formula: y = mx
m: 697.08552
x: Amount
y: Area

Figure C14. Calibration data for C 24:0 FAME

Raw data presented in Chapter 3:

Data used for the calculation of averages presented in Table 9 from Chapter 3:

Table C1. Raw data for the positive control (Direct Transesterification).

Replicate:	Mass of Biomass: (mg)	Mass of FAMEs: (mg)	% wt FAME:
1	100.50	11.50	11.45%
2	100.40	11.18	11.14%
3	100.70	10.94	10.87%
4	100.00	10.97	10.97%
5	99.70	10.87	10.90%
6	99.50	11.37	11.42%
Average:			**11.12%**

Table C2. Raw data for the wet algal biomass samples (Extracted Lipid Residue).

Replicate:	Mass of Biomass: (mg)	Mass of FAMEs: (mg)	% wt FAME:
1	98.94	6.81	6.88%
2	99.35	7.07	7.12%
3	99.96	7.48	7.49%
4	100.52	5.39	5.36%
5	100.83	7.07	7.02%
6	99.99	5.71	5.71%
Average:			**6.60%**

Table C3. Raw data for the wet algal biomass samples (Water Phase/Aqueous Phase).

Replicate:	Mass of Biomass: (mg)	Mass of FAMEs: (mg)	% wt FAME:
1	98.94	0.12	0.12%
2	99.35	0.13	0.13%
3	99.96	0.13	0.13%
4	100.52	0.12	0.12%
5	100.83	0.12	0.12%
6	99.99	0.13	0.13%
Average:			**0.13%**

Table C4. Raw data for the wet algal biomass samples (Precipitated Solid Phase).

Replicate:	Mass of Biomass: (mg)	Mass of FAMEs: (mg)	% wt FAME:
1	98.94	1.40	1.42%
2	99.35	1.58	1.59%
3	99.96	1.41	1.42%
4	100.52	2.61	2.59%
5	100.83	1.66	1.64%
6	99.99	2.68	2.68%
Average:			**1.89%**

Table C5. Raw data for the wet algal biomass samples (Residual Biomass).

Replicate:	Mass of Biomass: (mg)	Mass of FAMEs: (mg)	% wt FAME:
1	98.94	2.17	2.19%
2	99.35	2.39	2.41%
3	99.96	2.22	2.22%
4	100.52	2.36	2.35%
5	100.83	2.31	2.29%
6	99.99	2.26	2.26%
Average:			**2.29%**

Raw data presented in Chapter 4:

Data contained within this section relates to data presented in Chapter 4.

Table C6: Determination of moisture content of algal biomass used in study. R refers to the replicate number.

Sample:	Mass of Container:	Mass of wet algae:	Total Mass:	Post Drying Mass:	Mass of Dry Algae:	% Mass Change:	% Moisture:
105°C Samples							
105 WET R1:	13.3218	5.2374	18.5592	18.5592	5.2374	0.00%	81.30%
105 WET R2:	13.4166	4.9700	18.3866	18.3866	4.9700	0.00%	81.30%
105 WET R3:	13.4049	5.5346	18.9395	18.9395	5.5346	0.00%	81.30%
105 LYOPH R1:	13.2352	4.9524	18.1876	14.1661	0.9309	81.20%	0.09%
105 LYOPH R2:	13.3279	5.3865	18.7144	14.3351	1.0072	81.30%	0.00%
105 LYOPH R3:	13.5175	5.5208	19.0383	14.5451	1.0276	81.39%	-0.09%
				Average Moisture Content:		**81.30%**	
105 1H R1:	1.2849	5.0027	6.2876	3.6075	2.3226	53.57%	27.72%
105 1H R2:	1.2850	5.1125	6.3975	3.5268	2.2418	56.15%	25.15%
105 1H	1.2889	5.0553	6.3442	3.8915	2.6026	48.52%	32.78%

R3:							
105 2H R3:	1.2824	5.1768	6.4592	2.4332	1.1508	77.77%	3.53%
105 2H r1:	1.2868	5.0517	6.3385	2.4787	1.1919	76.41%	4.89%
105 2H r3:	1.2875	5.1630	6.4505	2.4084	1.1209	78.29%	3.01%
105 4H R1:	1.2831	4.9482	6.2313	2.2270	0.9439	80.92%	0.37%
105 4H R2:	1.2867	5.1014	6.3881	2.2616	0.9749	80.89%	0.41%
105 4H R3:	1.2839	4.9955	6.2794	2.2438	0.9599	80.78%	0.51%
105 8H R1:	1.2891	5.1610	6.4501	2.2643	0.9752	81.10%	0.19%
105 8H R2:	1.2860	5.0234	6.3094	2.2332	0.9472	81.14%	0.15%
105 8H R3:	1.2887	5.0474	6.3361	2.2500	0.9613	80.95%	0.34%
105 20H R1:	1.2869	5.0425	6.3294	2.2336	0.9467	81.23%	0.07%
105 20H R2:	1.2892	5.1871	6.4763	2.2615	0.9723	81.26%	0.04%
105 20H R3:	1.2827	5.0620	6.3447	2.2322	0.9495	81.24%	0.05%
105 32H R1:	1.2938	5.0257	6.3195	2.2354	0.9416	81.26%	0.03%
105 32H R2:	1.2869	5.0576	6.3445	2.2372	0.9503	81.21%	0.09%
105 32H R3:	1.2936	4.9911	6.2847	2.2233	0.9297	81.37%	-0.08%
85 Wet R1:	6.4584	6.1940	12.6524	12.6524	6.194	0.00%	88.69%
85 Wet R2:	6.4332	5.1180	11.5512	11.5512	5.118	0.00%	88.69%
85 Wet R3:	6.4464	5.6275	12.0739	12.0739	5.6275	0.00%	88.69%
85 Lyoph R1:	6.4454	5.5506	11.9960	7.0717	0.6263	88.72%	-0.02%
85 Lyoph R2:	6.4450	4.9597	11.4047	7.0061	0.5611	88.69%	0.01%
85 Lyoph	6.5108	5.3282	11.8390	7.1142	0.6034	88.68%	0.02%

R3:							
				Average Moisture Content:		**88.69%**	
85°C Samples							
85 1H R1:	1.2847	5.2760	6.5607	4.5740	3.2893	37.66%	51.04%
85 1H r2:	1.2855	5.0323	6.3178	4.5785	3.293	34.56%	54.13%
85 1H R3:	1.2890	5.0926	6.3816	4.3463	3.0573	39.97%	48.73%
85 2H R1:	1.2928	5.1792	6.4720	2.7725	1.4797	71.43%	17.26%
85 2H R2:	1.2930	5.1731	6.4661	2.7910	1.498	71.04%	17.65%
85 2H R3:	1.2918	5.0368	6.3286	2.7088	1.417	71.87%	16.83%
85 4H R1:	1.2801	5.0775	6.3576	1.8856	0.6055	88.07%	0.62%
85 4H R2:	1.2803	4.9803	6.2606	1.8687	0.5884	88.19%	0.51%
85 4H R3:	1.2788	5.0882	6.3670	1.8778	0.599	88.23%	0.47%
85 8H R1:	1.2847	5.1360	6.4207	1.8736	0.5889	88.53%	0.16%
85 8H R2:	1.2897	5.0495	6.3392	1.8719	0.5822	88.47%	0.22%
85 8H R3:	1.2917	5.0560	6.3477	1.8736	0.5819	88.49%	0.20%
85 20H R1:	1.2856	5.1235	6.4091	1.8670	0.5814	88.65%	0.04%
85 20H R2:	1.2912	4.9957	6.2869	1.8591	0.5679	88.63%	0.06%
85 20H R3:	1.2743	5.0375	6.3118	1.8465	0.5722	88.64%	0.05%
85 32H R1:	1.2865	5.0133	6.2998	1.8457	0.55922	0.888452716	-0.15%
85 32H R2:	1.2830	4.9876	6.2706	1.8487	0.5657	0.886578715	0.04%
85 32H R3:	1.2915	5.0934	6.3849	1.8682	0.5767	0.886775042	0.02%
Spare 1:	1.2817	5.0077	6.2894	4.4655	3.1838	36.42%	52.27%
Spare 2:	1.2855	5.0323	6.3178	4.5785	3.293	34.56%	54.13%
Spare 3:	1.2817	5.0023	6.2840	2.9462	1.6645	66.73%	21.97%
Spare 4:	1.2928	4.9978	6.2906	2.9330	1.6402	67.18%	21.51%
Spare 5:	1.2830	5.0544	6.3374	1.8834	0.6004	88.12%	0.57%
Spare 6:	1.2920	5.0292	6.3212	1.8910	0.599	88.09%	0.60%
65°C Samples							
65 Wet R1:	13.3891	5.4138	18.8029	18.8029	5.4138	0.00%	73.89%
65 Wet	13.3874	5.4040	18.7914	18.7914	5.4040	0.00%	73.89%

R2:							
65 Wet R3:	13.4190	5.4306	18.8496	18.8496	5.4306	0.00%	73.89%
65 Lyoph R1:	13.3876	6.1129	19.5005	14.9834	1.5958	73.89%	-0.01%
65 Lyoph R2:	13.3702	5.4553	18.8255	14.7945	1.4243	73.89%	0.00%
65 Lyoph R3:	13.3943	5.2423	18.6366	14.7635	1.3692	73.88%	0.01%
				Average Moisture Content:		**73.89%**	
65 1H R2:	1.2850	5.2427	6.5277	5.5744	4.2894	18.18%	55.71%
65 1H r1:	1.2833	5.4043	6.6876	5.5907	4.3074	20.30%	53.59%
65 1H r2:	1.2771	5.0569	6.3340	5.2034	3.9263	22.36%	51.53%
65 2H r1:	1.2820	5.1712	6.4532	4.4454	3.1634	38.83%	35.06%
65 2H r2:	1.2770	5.0131	6.2901	4.5415	3.2645	34.88%	39.01%
65 2H R1:	1.2805	5.1579	6.4384	4.3518	3.0713	40.45%	33.43%
65 4H R1:	1.2788	5.1639	6.4427	3.4693	2.1905	57.58%	16.31%
65 4H R2:	1.2758	5.2873	6.5631	3.7200	2.4442	53.77%	20.12%
65 4H R3:	1.2819	5.4278	6.7097	3.5754	2.2935	57.75%	16.14%
65 8H R1:	1.2808	5.2325	6.5133	2.7900	1.5092	71.16%	2.73%
65 8H R2:	1.2827	5.2873	6.5700	2.8049	1.5222	71.21%	2.68%
65 8H R3:	1.2735	5.2424	6.5159	2.7860	1.5125	71.15%	2.74%
65 20H R1:	1.2871	5.4249	6.7120	2.7940	1.5069	72.22%	1.67%
65 20H R2:	1.2808	5.5383	6.8191	2.8245	1.5437	72.13%	1.76%
65 20H R3:	1.2802	5.0222	6.3024	2.6862	1.4060	72.00%	1.88%
65 32H R1:	1.2757	5.4460	6.7217	2.7861	1.5104	72.27%	1.62%
65 32H R2:	1.2826	5.0950	6.3776	2.6917	1.4091	72.34%	1.55%
65 32H R3:	1.2780	5.2012	6.4792	2.7192	1.4412	72.29%	1.60%

Table C7: FAME production for each sample. Values presented within the Table G are mass of FAMEs generated in terms of % cell dry mass. R refers to the replicate number as stated in Table C6.

Sample:	Replicate 1:	Replicate 2:	Replicate 3:	Average:
105 WET R1:	4.70%	5.26%	5.18%	**5.05%**
105 WET R2:	4.96%	5.34%	2.85%	**4.38%**
105 WET R3:	5.28%	3.87%	4.86%	**4.67%**
105 LYOPH R1:	9.51%	9.35%	9.44%	**9.43%**
105 LYOPH R2:	9.95%	10.25%	9.77%	**9.99%**
105 LYOPH R3:	9.86%	9.79%	9.71%	**9.79%**
105 1H R1:	4.90%	3.53%	5.09%	**4.51%**
105 1H R2:	3.26%	3.18%	4.46%	**3.63%**
105 1H R3:	4.52%	4.74%	4.19%	**4.48%**
105 2H R3:	8.76%	9.02%	9.95%	**9.24%**
105 2H r1:	8.04%	8.28%	8.34%	**8.22%**
105 2H r3:	8.53%	8.57%	8.72%	**8.61%**
105 4H R1:	8.66%	8.98%	9.17%	**8.94%**
105 4H R2:	9.31%	8.99%	8.82%	**9.04%**
105 4H R3:	8.75%	9.00%	8.86%	**8.87%**
105 8H R1:	8.58%	8.79%	8.83%	**8.73%**
105 8H R2:	8.79%	8.68%	8.97%	**8.81%**
105 8H R3:	8.63%	8.68%	8.75%	**8.69%**
105 20H R1:	8.61%	8.84%	8.91%	**8.79%**
105 20H R2:	9.18%	8.61%	8.90%	**8.90%**
105 20H R3:	8.44%	8.69%	8.71%	**8.61%**
105 32H R1:	9.74%	8.61%	6.86%	**8.40%**
105 32H R2:	7.30%	8.58%	8.57%	**8.15%**
105 32H R3:	8.54%	8.01%	8.59%	**8.38%**
85 Wet R1:	2.20%	3.66%	3.57%	**3.14%**
85 Wet R2:	3.87%	4.31%	3.65%	**3.94%**
85 Wet R3:	3.85%	3.64%	3.67%	**3.72%**
85 Lyoph R1:	11.51%	11.28%	11.28%	**11.36%**
85 Lyoph R2:	11.24%	11.32%	11.23%	**11.26%**
85 Lyoph R3:	11.65%	11.55%	11.31%	**11.50%**
85 1H R1:	3.44%	3.53%	3.54%	**3.50%**
85 1H r2:	2.93%	3.30%	3.33%	**3.19%**
85 1H R3:	3.41%	3.38%	3.42%	**3.40%**
85 2H R1:	4.65%	5.00%	4.83%	**4.83%**

85 2H R2:	4.54%	3.96%	3.78%	**4.09%**
85 2H R3:	4.38%	4.24%	4.38%	**4.33%**
85 4H R1:	10.81%	11.59%	11.92%	**11.44%**
85 4H r1:	10.73%	11.95%	11.16%	**11.28%**
85 4H r2:	11.15%	10.97%	11.47%	**11.20%**
85 8H R1:	10.09%	11.33%	11.20%	**10.87%**
85 8H R2:	11.59%	11.79%	11.20%	**11.53%**
85 8H R3:	11.16%	11.37%	11.22%	**11.25%**
85 20H R1:	10.11%	11.46%	11.65%	**11.07%**
85 20H R2:	11.45%	11.49%	11.48%	**11.47%**
85 20H R3:	11.52%	11.47%	11.44%	**11.48%**
85 32H R1:	10.46%	11.58%	11.35%	**11.13%**
85 32H R2:	11.84%	11.56%	11.52%	**11.64%**
85 32H R3:	11.33%	11.48%	11.39%	**11.40%**
1H r 1	2.65%	2.99%	3.42%	**3.02%**
1H r 2	2.93%	3.30%	3.33%	**3.19%**
2H r1	4.55%	3.81%	4.17%	**4.18%**
2H r2	4.01%	4.03%	3.74%	**3.93%**
4H r1	10.73%	11.95%	11.16%	**11.28%**
4H r2	11.15%	10.97%	11.47%	**11.20%**
65 Wet R1:	4.99%	4.72%	4.57%	**4.76%**
65 Wet R2:	4.96%	5.11%	4.88%	**4.98%**
65 Wet R3:	4.82%	4.97%	4.92%	**4.90%**
65 Lyoph R1:	11.42%	11.54%	11.59%	**11.52%**
65 Lyoph R2:	11.37%	11.55%	11.57%	**11.50%**
65 Lyoph R3:	11.30%	10.97%	11.23%	**11.17%**
65 1H R2:	5.14%	5.59%	5.95%	**5.56%**
65 1H r1:	5.29%	6.21%	5.49%	**5.66%**
65 1H r2:	5.99%	6.12%	6.50%	**6.20%**
65 2H r1:	3.39%	4.01%	3.58%	**3.66%**
65 2H r2:	2.99%	3.75%	3.63%	**3.46%**
65 2H R1:	3.05%	2.88%	2.83%	**2.92%**
65 4H R1:	8.81%	9.13%	9.22%	**9.05%**
65 4H R2:	8.48%	8.81%	8.58%	**8.62%**
65 4H R3:	8.60%	8.50%	8.85%	**8.65%**
65 8H R1:	10.97%	10.90%	10.94%	**10.94%**
65 8H R2:	11.10%	10.91%	10.96%	**10.99%**
65 8H R3:	10.78%	10.84%	10.78%	**10.80%**
65 20H R1:	11.16%	11.04%	11.55%	**11.25%**
65 20H R2:	10.92%	10.84%	10.71%	**10.82%**
65 20H R3:	11.53%	10.76%	10.43%	**10.91%**

65 32H R1:	11.32%	10.81%	10.45%	**10.86%**
65 32H R2:	11.95%	11.54%	10.89%	**11.46%**
65 32H R3:	11.69%	10.71%	10.38%	**10.93%**

Table C8: Raw data for Figure 16 within Chapter 4. D.c.m. stands for dry cell mass. mL methanol solution is the acidified methanol added while acid concentration is (v/v) sulfuric acid in methanol.

Sample:	mL of Methanol Soln:	Acid Concentration:	Mass of Algae (mg):	% FAME by d.c.m.:
(1%/0.5) - 1	0.5	1.00%	99.8	0.67%
(1%/0.5) - 2	0.5	1.00%	99.9	0.60%
(1%/0.5) - 3	0.5	1.00%	100.5	0.79%
(1%/1.0) - 1	1	1.00%	99.6	6.10%
(1%/1.0) - 2	1	1.00%	99.8	5.75%
(1%/1.0) - 3	1	1.00%	99.5	6.16%
(1%/2.0) - 1	2	1.00%	100	8.29%
(1%/2.0) - 2	2	1.00%	99.9	8.77%
(1%/2.0) - 3	2	1.00%	99.8	8.40%
(1%/4.0) - 1	4	1.00%	100.4	8.80%
(1%/4.0) - 2	4	1.00%	100.2	8.99%
(1%/4.0) - 3	4	1.00%	99.7	8.33%
(2%/0.5) - 1	0.5	2.00%	100.2	7.17%
(2%/0.5) - 2	0.5	2.00%	100.4	7.19%
(2%/0.5) - 3	0.5	2.00%	100	6.22%
(2%/1.0) - 1	1	2.00%	100.3	9.55%
(2%/1.0) - 2	1	2.00%	100.4	9.81%
(2%/1.0) - 3	1	2.00%	99.8	9.78%
(2%/2.0) - 1	2	2.00%	100.4	9.69%
(2%/2.0) - 2	2	2.00%	99.7	9.35%
(2%/2.0) - 3	2	2.00%	100.2	9.35%
(2%/4.0) - 1	4	2.00%	100.3	9.50%
(2%/4.0) - 2	4	2.00%	100.3	9.78%
(2%/4.0) - 3	4	2.00%	100.3	9.74%
(5%/0.5) - 1	0.5	5.00%	100.4	9.50%
(5%/0.5) - 2	0.5	5.00%	100.3	10.43%
(5%/0.5) - 3	0.5	5.00%	100	10.35%
(5%/1.0) - 1	1	5.00%	100.3	10.26%
(5%/1.0) - 2	1	5.00%	100.1	10.57%
(5%/1.0) - 3	1	5.00%	100.3	10.14%

(5%/2.0) - 1	2	5.00%	100.4	10.41%
(5%/2.0) - 2	2	5.00%	100.3	10.08%
(5%/2.0) - 3	2	5.00%	100.3	10.19%
(5%/4.0) - 1	4	5.00%	100.5	10.55%
(5%/4.0) - 2	4	5.00%	100.6	9.81%
(5%/4.0) - 3	4	5.00%	100.3	9.84%
(5%/0.5) - 1	0.5	10.00%	100.3	10.29%
(5%/0.5) - 2	0.5	10.00%	99.8	10.14%
(5%/0.5) - 3	0.5	10.00%	100.2	9.56%
(5%/1.0) - 1	1	10.00%	100.7	9.73%
(5%/1.0) - 2	1	10.00%	100.8	10.65%
(5%/1.0) - 3	1	10.00%	99.8	10.09%
(5%/2.0) - 1	2	10.00%	100	9.53%
(5%/2.0) - 2	2	10.00%	99.5	10.57%
(5%/2.0) - 3	2	10.00%	99.6	11.95%
(5%/4.0) - 1	4	10.00%	100.2	10.62%
(5%/4.0) - 2	4	10.00%	99.9	11.40%
(5%/4.0) - 3	4	10.00%	99.8	10.37%

Table C9: Raw data for Figure 17 within Chapter 4. D.c.m. stands for dry cell mass. mL methanol solution is the acidified methanol added while acid concentration is (v/v) sulfuric acid in methanol.

Sample:	**mL of Methanol Solution:**	**Acid Concentration:**	**Mass of Algae (mg):**	**% FAME by d.c.m.:**
(1%/0.5) - 1	0.5	1.00%	101.94	0.48%
(1%/0.5) - 2	0.5	1.00%	101.89	0.30%
(1%/0.5) - 3	0.5	1.00%	99.49	0.43%
(1%/1.0) - 1	1	1.00%	102.2	1.29%
(1%/1.0) - 2	1	1.00%	103.08	1.19%
(1%/1.0) - 3	1	1.00%	101.07	1.18%
(1%/2.0) - 1	2	1.00%	102.43	3.22%
(1%/2.0) - 2	2	1.00%	100.8	3.20%
(1%/2.0) - 3	2	1.00%	99.7	3.56%
(1%/4.0) - 1	4	1.00%	99.69	4.97%
(1%/4.0) - 2	4	1.00%	102.2	4.14%
(1%/4.0) - 3	4	1.00%	97.11	5.36%
(2%/0.5) - 1	0.5	2.00%	99.33	0.42%
(2%/0.5) - 2	0.5	2.00%	101.49	0.45%
(2%/0.5) - 3	0.5	2.00%	102.55	0.48%

(2%/1.0) - 1	1	2.00%	101.94	3.01%
(2%/1.0) - 2	1	2.00%	99.7	2.17%
(2%/1.0) - 3	1	2.00%	100.43	1.97%
(2%/2.0) - 1	2	2.00%	101.78	5.23%
(2%/2.0) - 2	2	2.00%	101.12	5.04%
(2%/2.0) - 3	2	2.00%	102.97	5.23%
(2%/4.0) - 1	4	2.00%	99.09	7.74%
(2%/4.0) - 2	4	2.00%	101.01	7.35%
(2%/4.0) - 3	4	2.00%	100.15	7.80%
(5%/0.5) - 1	0.5	5.00%	99.69	1.38%
(5%/0.5) - 2	0.5	5.00%	102.2	1.49%
(5%/0.5) - 3	0.5	5.00%	97.11	1.47%
(5%/1.0) - 1	1	5.00%	101.07	4.11%
(5%/1.0) - 2	1	5.00%	102.25	4.38%
(5%/1.0) - 3	1	5.00%	99.98	4.31%
(5%/2.0) - 1	2	5.00%	101.38	7.01%
(5%/2.0) - 2	2	5.00%	101.54	6.71%
(5%/2.0) - 3	2	5.00%	100.17	7.24%
(5%/4.0) - 1	4	5.00%	101.38	8.90%
(5%/4.0) - 2	4	5.00%	101.54	9.07%
(5%/4.0) - 3	4	5.00%	100.17	8.93%
(5%/0.5) - 1	0.5	10.00%	99.01	2.12%
(5%/0.5) - 2	0.5	10.00%	102.78	2.01%
(5%/0.5) - 3	0.5	10.00%	98.62	2.49%
(5%/1.0) - 1	1	10.00%	98.65	5.19%
(5%/1.0) - 2	1	10.00%	102.28	5.20%
(5%/1.0) - 3	1	10.00%	99.7	5.20%
(5%/2.0) - 1	2	10.00%	100.39	7.77%
(5%/2.0) - 2	2	10.00%	101.64	7.57%
(5%/2.0) - 3	2	10.00%	102.68	7.51%
(5%/4.0) - 1	4	10.00%	101.07	8.93%
(5%/4.0) - 2	4	10.00%	102.25	8.50%
(5%/4.0) - 3	4	10.00%	99.98	9.08%

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