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Kinetics of Lipid Extraction from Microalgae

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DEDICATION

I would like to dedicate this thesis to my family, who has always been there to support me.

ACKNOWLEDGMENTS

ABSTRACT

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Kinetics of the Lipid Extraction from Microalgae
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The lipid extraction from microalgae is relatively energy-intensive, time-consuming and costly. Hexane is traditionally the extraction solvent of choice. However, hexane is toxic. Clearly it is highly desirable to develop a faster and environmentally safer microalgal lipid extraction technique. This is the focus of this project. The aims are: 1- Evaluate heptane as a substitute for hexane. Both are non-polar, but heptane is less toxic than hexane. Our data confirmed that hexane and heptane extract very similar material, so heptane is a possible replacement for hexane. 2- Collect experimental data on the rate of extraction of lipids from microalgae using hexane and heptane then use the data to determine the kinetics of the extraction process. A first order model was adopted, τ dY/dt = (K-Y), where Y is the g of lipid extracted per 100 g dry algae (initially at t = 0, Y = 0), K is the maximum g of lipid which can be extracted in the process per 100 g dry algae, t is the extraction time (min), and τ is the time constant for the process (min). Solving the differential equation gives $Y(t) = K\left[1 - e^{-t/\tau}\right]$. The maximum yield (K) and time constant (τ) depend on the solvent used and the solvent to dry algae ratio (R, mL of solvent used per g dry algae). The effect of the solvent to dry algae ratio (R) on the lipids extracted (Y) was studied. It was important to establish the validity of the first order kinetics model for both solvents and a large range of R. Microalgae was grown and harvested to supply enough dry algae for lipid extractions. Experimental data of Y vs. t were collected on a bench scale batch extractor, using hexane and heptane with R = 5 and 30. The extraction process was found to follow the assumed first order model. The maximum yield and time constant were determined from a least square error fit of the data to the proposed model. The results show that the diffusion of lipids from the algae into hexane is slow with time constants of 10-20 minutes. Heptane extraction was faster with time constants of 2- 7 minutes. For an R of 5, hexane's time constant was five times that of heptane (hexane τ was 10 min and heptane τ was 2 min). For an R of 30, hexane's time constant was a little less than triple that of heptane (hexane τ was 20 min while heptane τ was 7 min). These values confirm that the heptane extraction is faster than the hexane extraction. Increasing R from 5 to 30 (factor of 6), roughly doubled the time constant for hexane (10 min to 20 min), more than tripled the time constant for hexane (2 min to 7 min), increased hexane's maximum yield by 42% (2.75 to 3.90 g lipid extracted/100 g algae), and increased heptane's maximum yield by 45% (1.80 to 2.61 g lipid extracted/100 g algae). For R = 5 hexane's maximum yield was 53% larger than heptane's maximum yield (2.75 vs. 1.80 g lipid extracted/100 g algae). For R = 30, hexane's maximum yield was 49% larger than heptane's maximum yield (3.90 vs. 2.61 g lipid extracted/100 g algae). The results show that while hexane is slower at extracting the algae lipids, it extracts more lipids than heptane. Similarly, increasing the solvent to algae ratio (R) slows down the extraction, but increases the yield. Technically, heptane offers a potential safer alternative to hexane for microalgae oil extraction. From an engineering standpoint, further research is needed to determine if heptane is economically superior to hexane, and the optimum R.

NOMENCLATURE

C5	Pentane
C6	Hexane
C7	Heptane
$C_L(t)$	oil concentration in the solvent at any given time t [g/mL]
C_L^{*w}	final oil concentration (hypothetical) in the solvent phase due to the washing stage alone [g/mL]
C_L^{*d}	final oil concentration (hypothetical) in the solvent phase due to the diffusion stage alone [g/mL]
K	maximum g of lipid which can be extracted in the process per 100 g dry algae [g/g]
k_c^w	kinetics coefficient for the washing stage [1/min]
k_c^d	kinetics coefficient for the diffusion stage [1/min]
N	number of data points collected
R	solvent to dry algae ratio [mL:g]
SSE	sum of square errors [$(g/g)^2$]
t	extraction time (min)
Y	gram of lipid extracted per 100 g dry algae [g/g]
Y_i	gram of lipid extracted per 100 g dry algae at $t = t_i$ [g/g]
$Y(t_i)$	Value of the regression curve [g/g]
ε	error [g/g]
τ	time constant for the first order lipid extraction process (min)

CHAPTER 1

INTRODUCTION

1.1 Biodiesel

It is vital that scientists find clean, affordable, and renewable energy sources. Fossil fuels, our main source of energy, cause a great deal of pollution when burned, and the supply will not last forever. Biofuels, a renewable energy source, have the potential to help satisfy our energy needs. If biofuels become popular, there will be a reduction in fossil fuel usage and carbon dioxide emissions. Carbon dioxide is the most abundant greenhouse gas and leading cause of global warming. Global warming is serious, and if not addressed soon, the consequences could be disastrous for humans, animals, and the environment (Gelbspan 2001). Biofuels are a great way to reduce our dependence on fossil fuels and limit the destruction of the environment.

Biodiesel is an environmentally friendly alternative to petroleum diesel. Biodiesel is made from the transesterification of oils (such as grease, animal fats, vegetable oils, or algal oil) (GuanHua et al. 2010). The transesterification process follows this reaction:
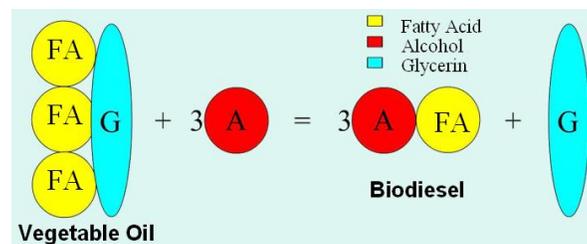
Figure 1.1: Traditional Transesterification Reaction to Produce Biodiesel

Vegetable oil is combined with an alcohol (ethanol or methanol) to produce biodiesel and glycerin. This reaction is slow; therefore, a catalyst is needed. The two most common catalysts are sodium hydroxide and potassium hydroxide. Biodiesel is the desired product, whereas glycerin is a by-product (Chaput et al. 2012, Chisti 2007 and 2008, Elmoraghy et al. 2012, Ferrentino 2007, Hoffman 2003, McConnell and Farag 2012, Mulumba 2010 and 2012, Webster 2010, Wilson et al. 2012, Woertz 2007 and Zuka et al. 2012).

1.2 Advantages of Biodiesel
- o Biodiesel is clean-burning and more environmentally friendly than petroleum diesel.
- o While the burning of biodiesel produces carbon dioxide emissions similar to fossil fuels, the plant or algae feedstock absorbs carbon dioxide from the atmosphere when it grows through photosynthesis. Therefore biodiesel is considered carbon neutral. In fact, biodiesel releases less greenhouse gases than diesel.
- o Biodiesel is biodegradable so it does not cause hazard to the environment when spilled (Biodiesel Handling and Use Guide 2008).
- o Biodiesel can be used in current diesel engines without any modifications.
- o Biodiesel can be produced from many different sources of oil, e.g., grease, vegetable oil, and animal fats. Therefore, biodiesel producers do not rely on one feedstock (Biodiesel Handling and Use Guide 2008). In the United States, soybean and canola oils are

commonly used to produce biodiesel (Wilson et al. 2012); however, in Europe, rapeseed oil is used. Rapeseed oil is the same as canola oil in the United States.

o It has economic advantages because it can be made in the US which reduces our dependency on imported petroleum (Demirbas 2011).

o Biodiesel can be used in blends with petroleum diesel. This is especially important in colder environments because biodiesel can gel in the cold weather, but when used in blends, gelling is not as much of an issue (Biodiesel Handling and Use Guide 2008).

o Biodiesel is a much better lubricant than conventional diesel fuel and extends engine life.

1.3 Disadvantages of Biodiesel

o Biodiesel is made from food plants. There is a great concern that if biodiesel production from food crops is increased substantially, many people will suffer from malnutrition (Brabeck-Letmathe 2008).

o Growing high volumes of crops requires fertile soil. Fertile soil is not available in all areas which means fertilizers are required. Fertilizers add to the cost to grow the crops which in turn increases the cost of biodiesel. If biodiesel is going to become a widespread fuel source, it must rival petroleum diesel prices. Also, the fertilizers can contaminate water supplies when they are washed away by rain or irrigation systems. Contaminated drinking water is a very serious problem that needs to be avoided (Cipiti 2007).

o If deforestation and monoculture farming techniques were used to grow biofuel crops, biodiesel is predicted to cause serious threats to the environment.

1.4 Microalgae as an Alternative Biodiesel Feedstock

Microalgae are simple, plant-like aquatic organisms that contain green pigments (chlorophyll) in the cells. The chlorophyll uses photonic energy (light), carbon dioxide (CO_2) and water to synthesize carbohydrates (energy storage) and make biomass (algae growth). Since algae are not human food sources, there is no dispute that increasing the production of biofuels will increase malnutrition. Land requirements must also be addressed when investigating biofuels. Microalgae are promising because they "can generate 15 times more oil per acre than other plants used for biofuels" ("Algae") and do not require arable (fertile) land to grow. Algae are much more versatile than plants. They can grow in salt water, freshwater or even contaminated water, at sea or in ponds, and on land not suitable for food production. Algae's versatility makes it an attractive alternative to food crops.

There are two main types of algae: microalgae and macroalgae. Microalgae are microscopic whereas macroalgae can be seen by the naked eye. An example of macroalgae is seaweed. This project focused on using microalgae to produce a biodiesel feedstock.

Research is being performed to explore microalgae growth in wastewater, (Greer et al. 2009, Woertz 2009, Craggs et al. 2011, Zuka et al. 2012, Chaput et al 2012, and Elmoraghy et al. 2012). Some studies looked at offshore algae growth in wastewater (McConnell et al. 2012). Humans produce a great deal of wastewater so it will help the environment if the water can be put to good use. In 2004, the United States alone produced approximately 35 billion gallons of wastewater per day. In 2004, the United States population was approximately 295 million. Therefore, approximately 120 gallons of wastewater per person per day was produced ("US Wastewater Treatment", "Population Estimates"). Also, if wastewater is used, then fresh drinking water will not be taken from those who need it. About one billion people have inadequate access to clean drinking water. Using wastewater is a good idea because it has most

of the nutrients that the algae need to grow, such as nitrates and phosphates. One crucial nutrient, carbon, is missing from the wastewater, but there is a simple solution to this problem. Carbon is added by bubbling air, which contains carbon dioxide, through the water. This will help reduce the amount of carbon dioxide in the atmosphere and minimize further global warming. Bubbling air through the water will not add much to production costs thus keeping the biodiesel prices low (Greer 2009). Clearly, microalgae do not have the land use, fresh water consumption, and food security impacts of other biofuels.

1.5 Photobioreactors

In order to grow microalgae, the cells must have nutrients, light, and carbon dioxide. Since the microalgae require a light source for photosynthesis, they must be grown in special bioreactors which allow light to penetrate the reactors walls and growth medium. If light cannot penetrate the bioreactor walls and growth medium, the cells will not grow. This added requirement means the bioreactor in which the microalgae are grown must be constructed of a clear material. These photobioreactors can be made of glass, plastic, or other materials which will allow light to penetrate. Also, the light source given to the cells does not need to be sunlight; artificial fluorescent lights are satisfactory for microalgae growth. Since fluorescent lights can be used to grow the microalgae, experiments can be performed in the laboratory with controlled light conditions (Webster 2010 and 2012, Eltringham et al. 2013).

1.6 Microalgae Growth

There are four phases of microalgae growth. When the algae inoculum is added to the photobioreactor, the algae go through a lag phase as they adjust to the new environment. During this phase, there is very little microalgae growth which can be seen in Figure 1.2. Once the microalgae have adjusted, they grow exponentially. Once the microalgae reach a maximum concentration, they enter a stationary phase in which the microalgae stop growing, but still continue to produce lipids. This is the optimal time to harvest the algae, because after the stationary phase the algae cells then enter the death phase and begin to die. Algae growth phases can be determined either by measuring the change in the turbidity over time or by performing cell counts.
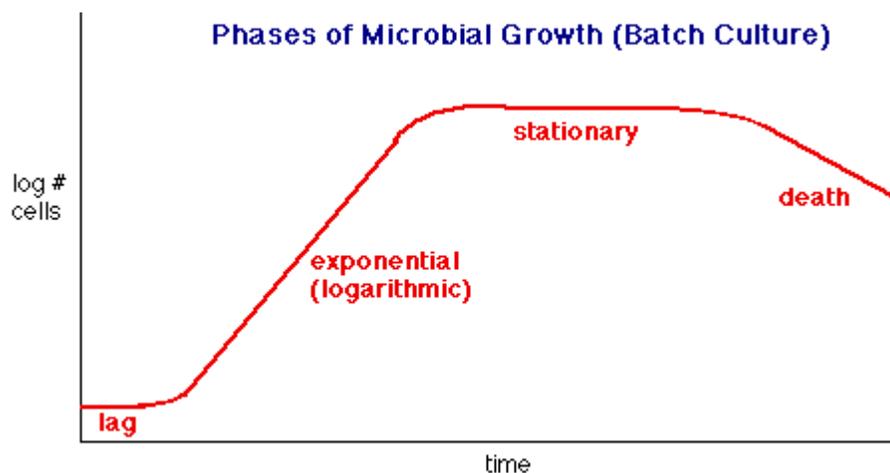
Figure 1.2: Microalgae Growth. There are four phases to microalgae growth: lag, exponential growth, stationary, and death. Microalgae harvesting is done during the stationary phase.

1.7 Microalgae Dewatering

Once the microalgae have reached the stationary phase, they will be dewatered. The microalgae must be dewatered because the lipid extraction process requires completely dry biomass. Centrifugation and freeze drying are commonly used to dewater the microalgae. Centrifugation removes the vast majority of water (roughly 90-95%), and freeze drying will remove the remaining water to produce dry algae.

1.8 Microalgae Lipid Extraction

Lipid extraction from dried microalgae is costly. Hexane is traditionally the extraction solvent of choice. However, hexane is toxic. It has to be heated until reflux and the extraction process can take 90 minutes or longer. This makes the process energy-intensive and time-consuming. To improve the process economics, it is important that the extraction process is environmentally safe and run for the optimal time. If the extraction time is too short, not enough lipids will be extracted, but if the extraction is too long, it may not be cost effective to continue heating the system. At this point, the variables which affect microalgae lipid extraction are not fully understood (Halim et al. 2012). This project will explore the kinetics of the extraction process to ensure that the optimal extraction time is used.

1.9 Project Significance

There have been many studies which analyzed microalgae growth, production of algae lipids, and biodiesel and Bio-Jet fuel production from microalgal lipids (Elmoraghy et al. 2012). However, commercial scale production of algae oil is still not cost-effective. Considerable research is taking place to trigger lipid formation in algae (Elmoraghy et al. 2012), and reduce the energy (Eltringham et al. 2013) and water requirements (Price et al. 2013, Elmoraghy 2013). One of the steps in the algae to biodiesel process is the extraction of oil from algae using hexane. This step has the following characteristics:

- o Hexane is very toxic to the peripheral nervous system.
- o Hexane is very volatile which leads to fugitive emissions and worker safety issues.
- o Solvent extraction is energy-intensive, time-consuming, and costly.

Clearly it is highly desirable to find a substitute solvent for the algae oil extraction process that would lower the toxicity, reduce fugitive emissions, improve worker safety, and reduce the extraction time.

1.10 Project Hypotheses

Several hypotheses form the basis of the current project:

- o The selected replacement solvent is a non-polar solvent like hexane (C6), and will exhibit the following characteristics: lower toxicity and faster extraction kinetics rate than hexane.
- o The solvent lipid extraction from algae is controlled by the diffusion of the lipids from the algae into the bulk of the solvent. There are no neutral lipids at the outer surface of the algae cells; hence there is no washing of lipids from the surface of the algae to the bulk of the solvent.
- o The kinetics of the lipid extraction follows a first order diffusion model.

1.11 Project Goals
The goals of this project were to:
- o Select a less toxic solvent for the algal lipid extraction process.
- o Investigate how the operating conditions affect the algal lipid extraction speed and yield.
- o Optimize the algal lipid extraction process.

1.12 Project Objectives
To accomplish the above goals the specific objectives of this project are given in Table 1.1.

Table 1.1: Project Objectives

No.	Objective Description
1	Literature review of algae lipid extraction data and kinetics models.
2	Select a solvent to replace hexane (C6).
3	Demonstrate that the selected solvent is a possible alternate to hexane (C6) in the lipid extraction.
4	Design a bench scale batch extraction apparatus to collect the data of extraction yield (Y) vs. extraction time (t).
5	Compare hexane (C6) and the selected solvent as the solvent used for the algae lipid extraction process.
6	Test the mathematical model which describes the diffusion/mass transfer involved in the algae lipid extraction and calculate the best fitted K and τ for both hexane (C6) and the selected solvent.
7	Examine the effect of the solvent to dry algae ratio (R) on the rate of algae lipid extraction. Operate with R values of 5 and 30 for both solvents.
8	Analyze data and present conclusions and recommendations for future work.

1.13 Thesis Organization
The material in this thesis is organized as follows:
- Chapter 2 – Literature Review
- Chapter 3 – Kinetics Modeling of Oil Extraction From Dry Microalgae
- Chapter 4 – Materials and Methods
- Chapter 5 – Results and Discussion
- Chapter 6 – Conclusions
- Chapter 7 – Recommendations for Future Work

CHAPTER 2

LITERATURE REVIEW

2.1 Microalgae Lipid Composition

The use of algae to produce biodiesel was studied by the US Department of Energy (Sheehan et al. 1988). Recently the production of microalgae biodiesel has expanded (Hu et al. 2008, Greenwell et al. 2010, Eltringham et al. 2013) and has spread to many countries (Khan et al. 2009). Microalgae have both polar and non-polar lipids. The non-polar lipids are the ones which are used to create biodiesel. Inside the microalgae cell, non-polar lipids are used for energy storage. Triglycerides-esters are the major components of oils. These consist of one molecule of glycerol and three molecules of fatty acids. The characteristics of an oil are usually described in terms of its fatty acids composition. Fatty acids have a carboxyl group which is polar and a hydrocarbon chain which is non-polar. Fatty acids are described by the number of carbons and double bonds in the hydrocarbon chain. If there is one double bond in the hydrocarbon chain, the fatty acid is termed monounsaturated. If there are two or more double bonds it is termed polyunsaturated. If there are no double bonds in the hydrocarbon chain, the fatty acid is considered saturated. The most common fatty acids in edible oils have 18 carbon atoms. These fatty acids include stearic, oleic, linoleic (2 double bonds) and linolenic (3 double bonds) acids.

The types of fatty acids produced by the microalgae cell depend on the conditions in which the cell was grown. Some of the factors which affect lipid production include nutrient composition and availability, temperature, light intensity, and aeration rate. Usually, the microalgal fatty acids have hydrocarbon chains ranging from 12 to 22 carbons. The extent of unsaturation can vary, but the number of double bonds never exceeds six. The length of the hydrocarbon chain and the degree of unsaturation influences the heating value, viscosity, cloud point, and pour point of the biodiesel which is created (Halim et al. 2012, Elmoraghy et al. 2013). Some of these effects are summarized in Table 2.1.

Table 2.1 Advantages and Disadvantages of Saturated and Unsaturated Fatty Acids

Fatty Acid Type	Advantages	Disadvantages
Saturated	Decreases NOx emissions Improves oxidative stability Reduces deposition	Increases melting point Increases viscosity Reduces lubricity
Unsaturated	Decreases melting point Decreases viscosity Improves lubricity	Increases NOx emissions Lowers oxidative stability Increases deposition

Table 2.2 (reproduced from Mulumba 2010) lists some saturated and unsaturated fatty acids found in algal cells. The name of each fatty acid is followed by the total number of carbon atoms, and the total number of double bonds; for instance, (16:1) indicates the fatty acid (palmitoleic or sapienic) has 16 carbon atoms with one double bond. J.-Y. Lee et al. 2010 reported the fatty acid composition of Chlorella vulgaris. The results are shown in Table 2.3. The most common fatty acid in Chlorella vulgaris is the unsaturated Linoleic acid, with two double bonds (18:2).

Table 2.2: Saturated and unsaturated FAs found in Algal cells (Matsumoto et al. 2009; Singh and Singh 2010)

Category	FA Name	Formula
Saturated	Capric (10:0)	CH_3-$(CH_2)_8$-$COOH$
Saturated	Lauric (12:0)	CH_3-$(CH_2)_{10}$-$COOH$
Saturated	Myristic (14:0)	CH_3-$(CH_2)_{12}$-$COOH$
Unsaturated	Myristoleic (14:1)	$CH_3(CH_2)_3\mathbf{CH=CH}(CH_2)_7COOH$
Saturated	Palmitic (16:0)	CH_3-$(CH_2)_{14}$-$COOH$
Unsaturated	Palmitoleic (16:1)	$CH_3(CH_2)_5\mathbf{CH=CH}(CH_2)_7COOH$ (= bond at C7)
Unsaturated	Sapienic (16:1)	$CH_3(CH_2)_8\mathbf{CH=CH}(CH_2)_4COOH$ (= bond at C10)
Unsaturated	Hexadecadienoic (16:2)	$CH_3(CH_2)_{10}\mathbf{CH=CHCH=CH}COOH$
Unsaturated	Hexadecatrienoic (16:3)	$CH_3(CH_2)_4\mathbf{CH=CH}CH2\mathbf{CH=CH}CH2\mathbf{CH=CH}$-$(CH_2)_2COOH$
Saturated	Stearic (18:0)	CH_3-$(CH_2)_{16}$-$COOH$
Unsaturated	Oleic (18:1)	$CH_3(CH_2)_7\mathbf{CH=CH}(CH_2)_7COOH$
Unsaturated	Linoleic (18:2)	$CH_3(CH_2)_4\mathbf{CH=CH}CH_2\mathbf{CH=CH}(CH_2)_7COOH$
Unsaturated	α-Linolenic (18:3)	$CH_3CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}(CH_2)_7$-$COOH$
Unsaturated	Octadecatetraenoic (18:4)	$CH_3CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}$ $CH_2\mathbf{CH=CH}(CH_2)_4$-$COOH$
Saturated	Arachidic (20:0)	CH_3-$(CH_2)_{18}$-$COOH$
Unsaturated	Arachidonic (20:4)	$CH_3(CH_2)_4\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH2$-$\mathbf{CH=CH}$-$(CH_2)_3COOH$
Saturated	Behenic (22:0)	CH_3-$(CH_2)_{20}$-$COOH$
Saturated	Eicosapentaenoic (20:5)	$CH_3CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH2$-$\mathbf{CH=CH}CH_2\mathbf{CH=CH}$ $(CH_2)_3COOH$
Unsaturated	Erucic (22:1)	$CH_3(CH_2)_7\mathbf{CH=CH}(CH_2)_{11}COOH$
Unsaturated	Docosapentaenoioc (22:5)	$CH_3CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH_2$-$\mathbf{CH=CH}CH2$-$\mathbf{CH=CH}$-$(CH_2)_3COOH$
Unsaturated	Docosahexaenoic (22:6)	$CH_3CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH_2$-$\mathbf{CH=CH}CH_2$-$\mathbf{CH=CH}CH_2\mathbf{CH=CH}(CH_2)_2COOH$
Saturated	Lignoceric (24:0)	CH_3-$(CH_2)_{22}$-$COOH$

Table 2.3: Fatty Acid Composition for Chlorella vulgaris (J.-Y. Lee et al. 2010)

Category	FA Name	Formula	Weight %
Saturated	Heptadecanoic/ Margaric (17:0)	CH_3-$(CH_2)_{15}$-$COOH$	0.1
Saturated	Stearic (18:0)	CH_3-$(CH_2)_{16}$-$COOH$	3.4
Unsaturated	Oleic (18:1)	$CH_3(CH_2)_7\mathbf{CH=CH}(CH_2)_7COOH$	16.3
Unsaturated	Linoleic (18:2)	$CH_3(CH_2)_4\mathbf{CH=CH}CH_2\mathbf{CH=CH}(CH_2)_7COOH$	79.4
Unsaturated	α-Linolenic (18:3)	$CH_3CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}(CH_2)_7$-$COOH$	0.1

2.2 Algae Harvesting

Algae harvesting is the process of separating microalgae from the growth medium and drying the microalgae completely. The biggest problem is that the microalgae concentration is not very high (too high of a concentration greatly reduces the light penetration to the middle of the photobioreactor). This means a significant amount of water must be removed in the harvesting process. There are many ways to separate the cells and water, each with their own advantages and disadvantages. Some processes include: using microscreens, centrifugation, flocculation, and filtration. High speed centrifugation (5000 revolutions per minutes) is commonly used because it does not require flocculants to settle the algae, and it is a relatively easy process. After centrifugation, the microalgae cells are freeze dried (lyophilized) to completely dry the microalgae to a powder. The freeze drying process takes about 2 days to complete (Ferrentino 2007, Mulumba 2010 and 2012, Chaput et al. 2012, Elmoraghy 2012 and 2013, and Price et al. 2013). J.-Y. Lee et al. 2010, used centrifugation and overnight freeze drying (at -70 °C under a vacuum) to harvest their algae. Once the dry algae have been obtained the lipids can be extracted.

2.3 Algae Lipid Extraction

Halim et al. 2012 proposed a five step mechanism for the solvent lipid extraction from microalgae:

1. The solvent penetrates the cell membrane and enters the cytoplasm. The cytoplasm is that part of the cell between the cell membrane and the nuclear envelope. This jelly-like substance is where the functions for cell expansion, growth, metabolism, and replication are carried out.
2. The non-polar solvent interacts with the non-polar lipids in the cytoplasm. Since both molecules are non-polar the interactions are van der Waals forces.
3. The solvent and lipid form a complex.
4. The solvent-lipids complex will diffuse out of the cell because of concentration driving force (gradient).
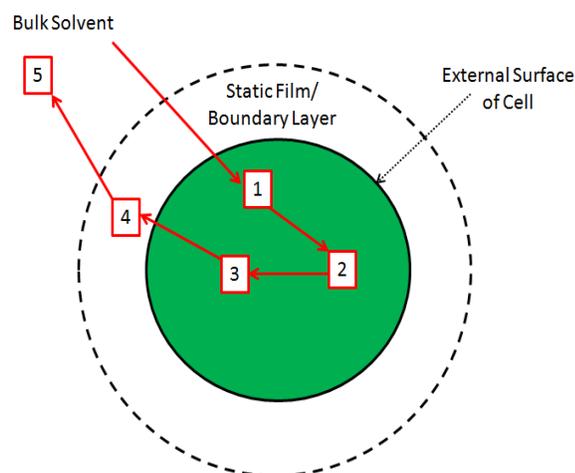5. The complex moves through the static film surrounding the cell and enters the bulk solvent.

Figure 2.1: Lipid Extraction Mechanism

2.4 Hexane Solvent Substitution

Hexane is an alkane hydrocarbon with the chemical formula $CH_3(CH_2)_4CH_3$ or simply C_6H_{14}. It used to be called Petroleum Naphtha fraction. It is an inert non-reactive and non-polar solvent. This makes it an "ideal" solvent to extract edible oils. The substitute solvent should satisfy a number of criteria, listed in Table 2.4.

Table 2.4: Hexane Solvent Substitution Criteria

Criteria	Explanation
Cost	Less than or comparable to hexane
Effectiveness	Higher or comparable extracted lipid yield (Y) than hexane
Extraction time	Shorter than or comparable to hexane extraction time
Safety	Safer or less toxic than hexane
Equipment	Drop-in solvent that requires no equipment modifications
Polarity	Non-polar solvent to minimize extraction of water soluble material and water soluble pigments

Ayers and Dooley (1948) experimented with 14 different hydrocarbon solvents in the C5-C7 range. They looked at the effectiveness at extracting cottonseed oil. They recommended avoiding aromatic and highly branched solvents. They suggested substitution of methyl heptane for hexane as a solvent to extract cottonseed oil. While hexane extraction gave better quality oil, methyl heptane was faster in the extraction.

Pons and Eaves (1967) compared 4 solvents (acetone, butanol, ethyl ether, and benzene) to hexane in the extraction of cottonseed oil under identical conditions. Acetone, being polar solvent, resulted in darker oil than hexane. They concluded that hexane was superior to the 4 alternatives. This was further confirmed by Taha et al. (1988) who studied the solvent extraction of oil from cottonseed.

MacGee (1937) studied the solvent extraction of oil from oilseeds. The factors he considered were oil stability, odor, taste, low evaporation losses of solvent, and lack of erosion in the extraction equipment. He recommended the use of the narrow petroleum boiling range solvents, hexane and heptane. Ayers and Dooley (1948) used hexane and heptane to extract cottonseed oil in the lab. They concluded that while hexane and heptane have similar extracted oil yields, the quality of the hexane extracted oil was better. Seher et al. (1958) noted that heptane extracted more phospholipids than hexane. Conkerton et al. (1995) did a lab scale study on replacing hexane with heptane in the extraction of oil from cottonseed using a solvent to meal ratio of 10. They noted that the yield (Y) and quality of the extracted oil was very similar for both solvents. Heptane extracted oil, however, required a higher temperature and longer time to be desolventized than the hexane extraction. This was in agreement with earlier results of Johnson and Lusas (1983). They concluded that heptane offers a potential alternative to hexane for oil extraction from cottonseed.

2.5 Extraction Kinetics Modeling

Patricelli et al. (1979) proposed a mathematical model for the extraction of oil from rapeseed in a batch reactor. The equation is derived from mass balances, solid-liquid equilibrium, and mass transfer rate expressions. The model gives the concentration of oil in the solvent over

time. It involves two simultaneous processes, washing and diffusion. Both of these processes have a different kinetic coefficient. Patricelli's model is:

$$C_L(t) = C_L^{*w}(1 - \exp[-k_c^w t]) + C_L^{*d}(1 - \exp[-k_c^d t]) \tag{1}$$

where $C_L(t)$ is the oil concentration in the solvent at any given time t, C_L^{*w} is the final oil concentration (hypothetical) in the solvent phase due to the washing stage alone, C_L^{*d} is the final oil concentration (hypothetical) in the solvent phase due to the diffusion stage alone, k_c^w is the kinetics coefficient for the washing stage, and k_c^d is the kinetics coefficient for the diffusion stage. The microalgae lipid extraction does not have a washing step because all of the algal lipids are contained within the cell. Since the original model was the sum of the separate washing and diffusion stages, the washing term can be dropped. The simplified expression is

$$C_L(t) = C_L^{*d}[1 - \exp(-k_c^d t)]. \tag{2}$$

Equation (2) is in the same form as a first order process. In order to make this point clear and for the ease of use, Eqn. (2) was written differently. The kinetics model which will be used in this thesis is:

$$Y(t) = K\left[1 - e^{-t/\tau}\right] \tag{3}$$

where Y is the lipid yield (grams of lipid extracted per 100 g dry algae), K is the maximum yield (grams of lipid extracted per 100 g dry algae) which can be obtained in the process with the given solvent, t is the extraction time, and τ is the time constant for the process. If the maximum yield (K) is large, lots of lipids will be extracted and the solvent is very effective. If the maximum yield (K) is small, a small amount of lipid will be extracted and the solvent is not very effective. If the time constant (τ) is large, the process is slow and it will take a long time for the yield to reach its maximum. If the time constant (τ) is small, the process is fast and it will take a short time for the yield to reach its maximum.

Pai and Lai (2011) studied algae growth and oil production in wastewater in a batch reactor. They cultured algae in a one-liter cylindrical photobioreactor (PBR) equipped with a magnetic stirrer and a light source next to the PBR. The illumination intensity was 2300 Lux, and the temperature was 30°C. They used first order reaction equations to model the algae growth and oil production. Their batch runs lasted 7 days hence they did not observe the stationary phase of algae growth (Figure 1.2).

2.6 Literature Review Conclusion

The literature review supports the objectives of this research. There is a need for understanding the extraction of oil from microalgae, selecting a safer non-polar extraction solvent, developing a kinetic model of algae oil extraction in a batch reactor, and comparing a the two solvents for safety, rate and yield of oil extraction, and cost. These are addressed in this investigation.

CHAPTER 3

KINETICS MODELING OF OIL EXTRACTION FROM DRY MICROALGAE

A first order model is proposed to describe the kinetics of the lipid extraction from algae. The model assumes a mass transfer/diffusion mechanism. The proposed model is derived from basic principles.

3.1 Derivation of Mathematical Model

The mass transfer of lipids during solvent extraction can be described by a first order model (Ozkal et al. 2005). The kinetics model equation is

$$\tau \, dY/dt = (K-Y)$$

where Y is the g of lipid extracted per 100 g dry algae (initially at time, t= 0, Y = 0), K is the maximum g of lipid which can be extracted in the process per 100 g dry algae, t is the extraction time (min), and τ is the time constant for the process (min). This model is a lumped form of Fick's Law of Diffusion (Halim et al. 2012).

Solving the differential equation gives:

$$Y(t) = K\left[1 - e^{-t/\tau}\right]. \tag{3}$$

The maximum yield (K) and time constant (τ) depend on the solvent used and the ratio (R, ml of solvent used per g dry algae used). This equation matches Patricelli et al. (1979) model without a washing stage.

3.2 Mathematical Model and Physical Situation

The model matches the physical situation. Figure 3.1 shows an example plot of the microalgae lipid extraction model. In the beginning, there is a large concentration gradient between the lipids inside the microalgae cell and in the bulk solvent. This means that the extraction rate is fast in the beginning of the extraction which matches the large slope at the beginning of the model. As time progresses, the concentration gradient decreases and the rate slows. This is seen in the model as the curve starts to level off. The microalgae have a finite amount of lipids, so when the extraction time is much larger than the time constant, increases in the extraction time do not change the yield. This is shown in the model when the curve approaches a horizontal asymptotic value at long extraction times.
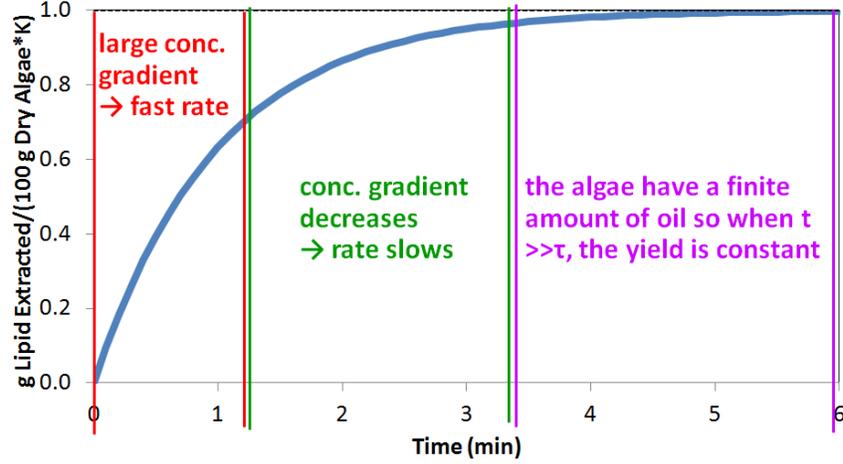
Figure 3.1: Example Plot of the Microalgae Lipid Extraction Model.

3.3 Relating the Kinetic Parameters to Experimental Data

Equation (3) can be written in the form of a straight line,

$$\frac{-t}{\tau} = \ln\left(1 - \frac{Y(t)}{K}\right). \tag{4}$$

However, it is not possible to use Eqn. (4) for curve fitting; the sum of square errors curve fitting approach must be used. The sum of square errors (SSE) is defined as

$$\sum error^2 = \sum[Y_i - Y(t_i)]^2 = SSE. \tag{5}$$

where Y_i is the lipid yield obtained for an extraction of time t_i, and $Y(t_i)$ is the value of the regression curve. Substituting the equation for $Y(t_i)$ in Eqn. (5) gives

$$\sum \varepsilon_i^2 = \sum\left[Y_i - K(1 - e^{-t_i/\tau})\right]^2 \tag{6}$$

To obtain the best curve fitting, the SSE must be minimized. To reduce the SSE, the partial derivative of the SSE with respect to K and $1/\tau$ must be equal to 0. The derivative of the SSE with respect to K is

$$\frac{\partial SSE}{\partial K} = \sum_{i=1}^{N} 2\left(Y_i - K\left(1 - e^{-t_i/\tau}\right)\right)\left(e^{-t_i/\tau} - 1\right). \tag{7}$$

where N is the number of data points. Since $\frac{\partial SSE}{\partial K} = 0$, Eqn. (7) can be simplified down to

$$K = \frac{\sum_{i=1}^{N} Y_i(1 - e^{-t_i/\tau})}{\sum_{i=1}^{N}(1 - e^{-t_i/\tau})^2}. \tag{8}$$

The partial derivative of the SSE with respect to $1/\tau$ is

$$\frac{\partial SSE}{\partial\left(\frac{1}{\tau}\right)} = \sum_{i=1}^{N} 2\left(Y_iK + Ke^{-t_i/\tau}\right)\left(-Kt_ie^{-t_i/\tau}\right). \tag{9}$$

Since $\frac{\partial SSE}{\partial\left(\frac{1}{\tau}\right)} = 0$, Eqn. (9) can be simplified down to

$$\sum_{i=1}^{N} Y_i t_i e^{-t_i/\tau} = \frac{\sum_{i=1}^{N} Y_i\left(1-e^{-t_i/\tau}\right)}{\sum_{i=1}^{N}\left(1-e^{-t_i/\tau}\right)^2} \sum_{i=1}^{N} t_i\, e^{-t_i/\tau}\left(1 - e^{-t_i/\tau}\right). \tag{10}$$

3.4 Evaluation of Kinetics Parameters

Equations (8) and (10) are two simultaneous, non-linear, algebraic equations in the Kinetics model parameters. There is no obvious algebraic manipulation of the equations to obtain two separate explicit equations. The experimental yield data for the extraction of microalgae oil is collected using a bench scale, batch extraction unit. The data are reported as the instantaneous yield, Y_i at time t_i and entered into Microsoft Excel. Excel calculates all the required summations in Equations (8) and (10). Values of the time constant are guessed until the difference between the left and right sides of Equation (10) is minimized and the fitted line describes the data. Equation (8) is used to calculate the maximum yield.

CHAPTER 4

MATERIALS AND METHODS

4.1 Algae Species Being Grown and Growth Requirements

Chlorella vulgaris was grown in a nutrient medium containing macronutrients and micronutrients. Chlorella vulgaris is a green alga which is very resilient; it can be grown in wastewater. Reverse Osmosis (RO) water was used to create the nutrient mediums in this project. The composition of the nutrient solution is listed in Appendix A. Nutrients were added to the RO water and then mixed until uniform. The algae inoculum (200 mL) was added to 80 L of nutrient medium. The same nutrient medium was used for all algae growth trials. The photobioreactor had a volume of 89 L, was cylindrical in shape, and made of clear plastic. The temperature was between 25°C and 27°C. Fluorescent lighting and aeration were provided continuously throughout the growing phase.

4.2 Turbidity Measurements

Analyzing the growth of microalgae was not the focus of this project. However, the microalgae growth was monitored so that the microalgae were harvested when its neutral lipid content was the highest, at the end of the stationary phase. Turbidity measurements using a Bosch and Lomb Spectronic 21 spectrophotometer (shown in Fig. 4.1) were one method used to monitor the microalgae growth. The light wavelength from the spectrophotometer was set to 680 nm to ensure that the measurement was sensitive to changes in cell concentration. Turbidity and light absorbance can be used to track microalgae growth because the measurement is proportional to the concentration. By measuring the turbidity every day the growth curve, like that in Fig. 1.2, could be generated and it would be easy to determine when the microalgae should be harvested. The term turbidity is used when the fluid is colored because of suspended solids. So, when using the spectrophotometer and the algae samples, the result is a turbidity measurement. When the color is due to a molecule in the fluid, I call it absorbance. So, the oil measurements reported in the Results Chapter are called absorbance.

Figure 4.1: Bosch and Lomb Spectronic 21 spectrophotometer used for turbidity measurements during this projects.

4.3 Cell Counting

Another method of monitoring algae growth was to manually perform a cell count using a hemocytometer. This was done by taking a sample of the algae from the 80L tank, making the appropriate dilution (so counting cells was manageable), and placing a sample on the hemocytometer. The hemocytometer is divided into 4 large squares that are broken in 16 smaller squares. Each of the 16 small squares in Box A or C has a volume of 0.00025 mm^3. Cells were counted in 12 of the small squares around the perimeter of either Box A or C. The average number of cells in each small square was calculated. This average cell count was then divided by the volume of a small square to get the concentration of cells per volume. By performing cell counts every day, a plot of cell concentration vs. time could be generated. The shape of this curve should be the same as the shape of the turbidity vs. time curve. Figure 4.2 shows the boxes used for the cell counting.
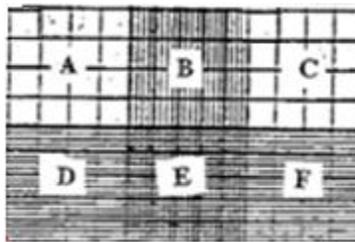

Figure 4.2: Diagram of the Squares on the Hemocytometer used for Cell Counts.

4.4 Algae Harvesting

A Damon/IEC B-20A centrifuge was used to remove the majority of the water from the algae solution. The medium was be spun in the centrifuge at 5000 rpm for 10 minutes. After centrifugation, the samples were freeze dried at -80°C under vacuum for 48 hours using a Labconco Freeze Dryer 5. At the end of the freeze drying process, the algae were completely dry. Four batches of 80 L nutrient mediums were grown for this experiment. Each batch produced about 50 g of algae. The 200 g of algae were combined in one container and mixed to obtain one homogenous mixture. Algae samples were taken from this mixture for the lipid extraction experiments.

4.5 Lipid Extraction Process

4.5.1 Experimental Setup (Objective 4)

The lipid extractions in this project used the work of Ferrentino 2007 as a template. Ferrentino used dry algae powder and a soxhlet extractor. Ferrentino heated hexane at reflux (70°C) for 5 hours. The dry algae powder was contained between two layers of glass wool inside the soxhlet. The hexane removes the lipids from the dry algae and returns back to the hexane reboiler at the bottom of the unit. The glass wool holds the algae inside the soxhlet and prevents any solids from entering the reboiler. After the extraction was completed, Ferrentino evaporated the hexane and obtained dry lipids. He reported the lipid content, as the mass of the dry lipids per mass of dry algae.

This project did not use a soxhlet extractor. Instead, the dry algae was put directly into the solvent and heated under reflux inside a round bottom flask. A hot water bath was used because it makes controlling the temperature of the extraction easy and it ensured uniform heating. The hot water bath sat on a combined hot plate and stirrer. Both the hot water bath and

solvent mixture was mixed throughout the extraction. The round bottom flask had three ports. The water cooled condenser was connected directly to the round bottom flask using one of the ports. The other ports could be used to take samples or insert/remove a stir bar. Since the condenser was not closed at the top, the system was at atmospheric pressure. Operating at a low pressure reduced the risk of breaking glassware or other accidents. Figure 4.3 shows the extraction setup.

Figure 4.3: Experimental Setup for the extraction of microalgal lipids using either hexane or heptane.

4.5.2 Experimental Procedure

The first step in the extraction process is to grind the dry algae into a fine powder. During the freeze drying process the algae can clump and the clumps do not always break apart when the algae is put into the solvent. The extraction rate depends significantly on the surface area of cells exposed to the solvent. If there are big clumps, the surface area will be decreased. The majority of extractions completed for this project were done with 5.00 g of algae. This amount of algae was large enough so that there were not inaccuracies with mass measurements, but not so large that we used up the algae supply quickly. The solvent to algae ratio determined the amount of solvent to use once the algae had been weighed out. For this project, solvent (mL) to algae (g) ratios of 5:1 and 30:1 were tested. The solvent, algae, and stir bar were combined in the round bottom flask and heated for either 1 minute, 4 minutes, 30 minutes, or 60 minutes. After the extraction time was up, the round bottom flask was removed from the hot water bath and allowed to cool. Cold water was running through the condenser for the entire extraction and cool down processes.

Once the round bottom flask cooled down, the algae cells were removed using filtration. Whatman #5 filter papers were used for the filtrations. After filtering, the lipids and solvent were in a flask. Then, the solvent was evaporated which left the lipids in the flask The mass of lipids recovered could be determined and used to calculate the extraction yield. Figure 4.4 shows the steps required to recover the extracted lipids.
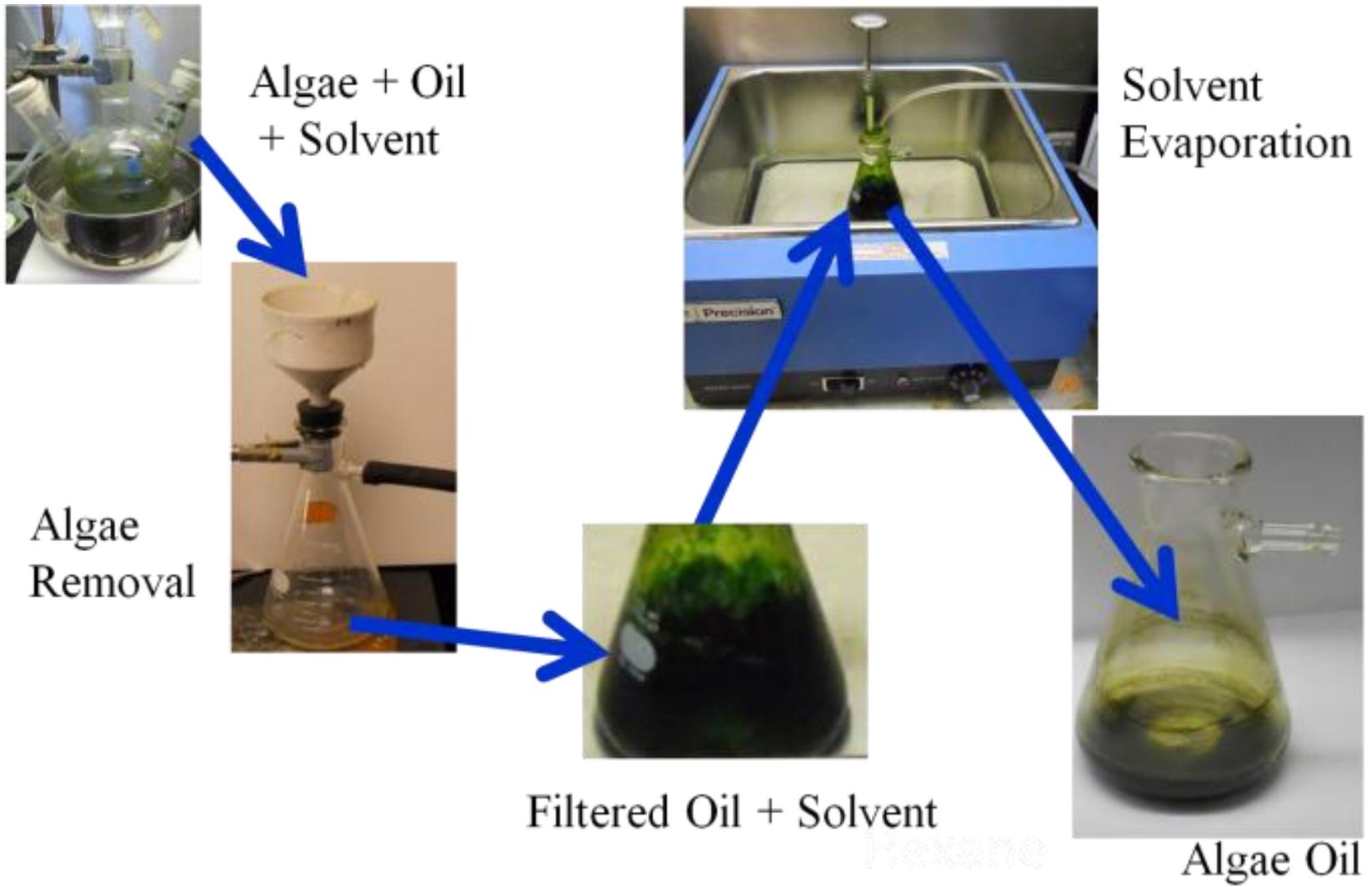
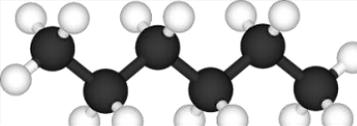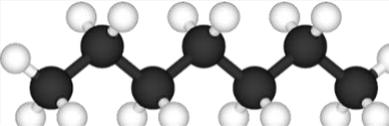Figure 4.4: Steps required to recover the extracted lipids.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Solvent Selection (Objective 2)

Based on literature survey, heptane was chosen because it is only one carbon chain longer than hexane. Also, hexane and heptane are both non-polar solvents. Table 5.1 shows the physical properties for hexane and heptane. Hexane is very toxic to the peripheral nervous system whereas heptane is only slightly toxic. This is a major advantage for heptane. Heptane has a boiling point of 98°C at 1 atm which is 30°C greater than hexane's boiling point. Using a solvent with a high boiling point is advantageous because diffusion is faster at higher temperatures and the cell membranes are more likely to break spilling out the lipids. Since heptane is less volatile than hexane, there will be less fugitive emissions from equipment. The energy required to bring the solvents from 20°C to their boiling point, and then vaporize them is roughly the same. Finally, when purchased on an industrial scale, the costs for hexane and heptane are comparable.

Table 5.1: Properties of Hexane and Heptane (Takueshi et al. 1980)

Property	Hexane, C_6H_{14}	Heptane, C_7H_{16}
Chemical Structure; Both are non-polar solvent		
Toxicity to the peripheral nervous system	very toxic	slightly toxic
Boiling Point (1 atm)	68°C	98 °C
Vapor Pressure (25 °C)	17.3 kPa	5.3 kPa
Energy Required to Boil from 20ºC	475 kJ/kg	493 kJ/kg
Cost, (Industrial Scale), $/gallon	1.15-1.19	1.21-1.64
Algae Lipid Extraction Effectiveness	Very Effective	To be determined

5.2 Heptane Viability (Objective 3)

The second task in the laboratory was to determine if heptane was a viable substitute for hexane. Based on the literature review and the comparison of the physical and chemical properties, heptane seemed like a good candidate, but it had to be proven in the laboratory. First, a lipid extraction was performed with hexane. Instead of completely evaporating the solvent, a portion of the solvent-lipid mixture was analyzed in the spectrophotometer. The absorbance of the sample was measured at wavelengths ranging from 340 nm to 970 nm. At each wavelength, the machine was zeroed with a sample of pure hexane so the absorbance measurement was due only to the lipids in the hexane. The plot of absorbance (scaled from 0 to 1) versus wavelength is shown in Fig. 5.1. There was a major absorbance peak at 410 nm, and a minor absorbance peak at 640 nm.
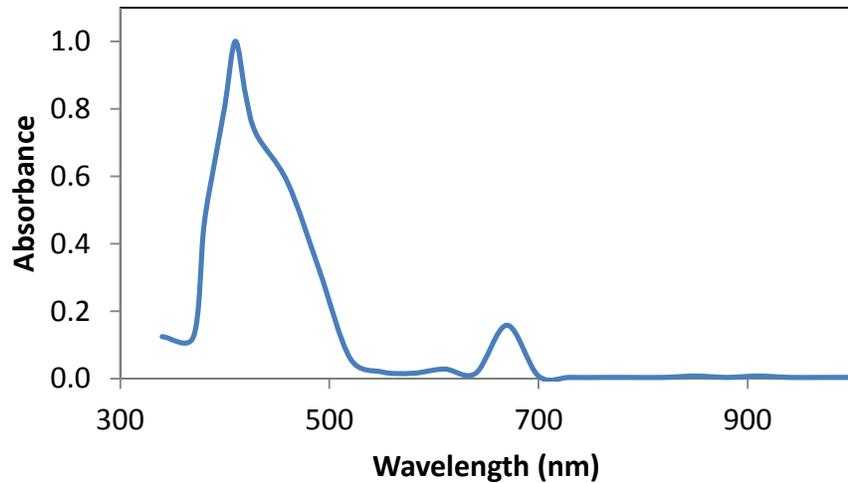
Figure 5.1: Absorbance versus Wavelength for Lipids Extracted using Hexane.

After the hexane extraction, another extraction was performed with heptane. The procedure was same for this extraction. Figure 5.2 shows the plots of absorbance (scaled from 0 to 1) versus wavelength for both heptane and hexane. Heptane had a major absorbance peak at 410 nm and a minor absorbance peak at 660 nm. The absorbance versus wavelength plots for hexane and heptane were very similar. They were close enough that it was reasonable to assume that hexane and heptane extract the same types of lipids. Since heptane had been confirmed as a possible substitute for hexane, the project was able to advance to the analysis of the kinetics of the lipid extraction from microalgae using hexane and heptane solvents.
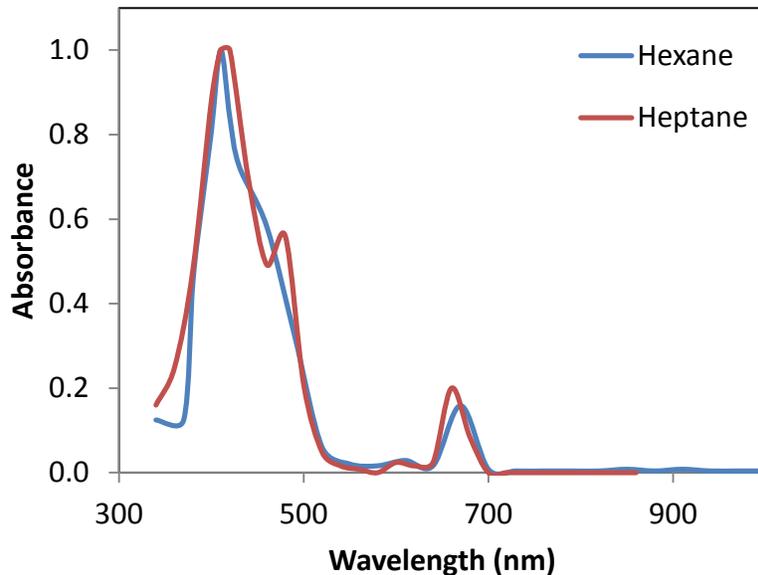

Figure 5.2: Absorbance versus Wavelength for Lipids Extracted using Hexane and Heptane.

5.3 Kinetics Model Parameters (Objectives 5 and 6)

      Figure 5.3 shows the first order extraction curve for the hexane solvent extraction of microalgal lipids at a solvent to algae ratio of 5:1. The rate of extraction is very quick at the beginning of the process. At four minutes, the yield is 1.2 g lipid extracted per 100 g of dry algae. The maximum yield is 2.75 g lipid extracted per 100 g of algae; so at the four minute mark, the extraction is already 36% complete. At 30 minutes the yield is 2.56 g lipid extracted per 100 g of algae which is 93% of the maximum. At 60 minutes, the extraction has basically gone to completion. Performing the extraction for longer than 60 minutes probably does not make economic sense. The energy required to continue the extraction probably exceeds the energy contained in the extra lipids which could be extracted. The data for this extraction (hexane at 5:1) fits the model well. The time constant was determined to be 10 minutes.

Figure 5.3: First Order Extraction Curve for the Hexane solvent extraction of microalgal lipids at a solvent to algae ratio of 5:1.

      Figure 5.4 shows the first order extraction curve for the hexane solvent extraction of microalgal lipids at a solvent to algae ratio of 30:1. The rate of extraction is fastest at the beginning of the process. Over time, the rate gradually slows until leveling off around 100 minutes. The measured yield at one minute was higher than the measured yield at four minutes. This is due to experimental error. The maximum yield is 3.90 g lipid extracted per 100 g of dry algae. At 30 minutes, the yield is 2.64 g lipid extracted per 100 g of dry algae which is 68% of the maximum. At 60 minutes, the extraction is close to completion. There appears to be some incentive to continue the extraction to 100 minutes, but beyond 100 minutes, the energy return is smaller than the energy invested in the extraction process. The data for this extraction (hexane at 30:1) fits the model satisfactorily. The time constant was determined to be 20 minutes.
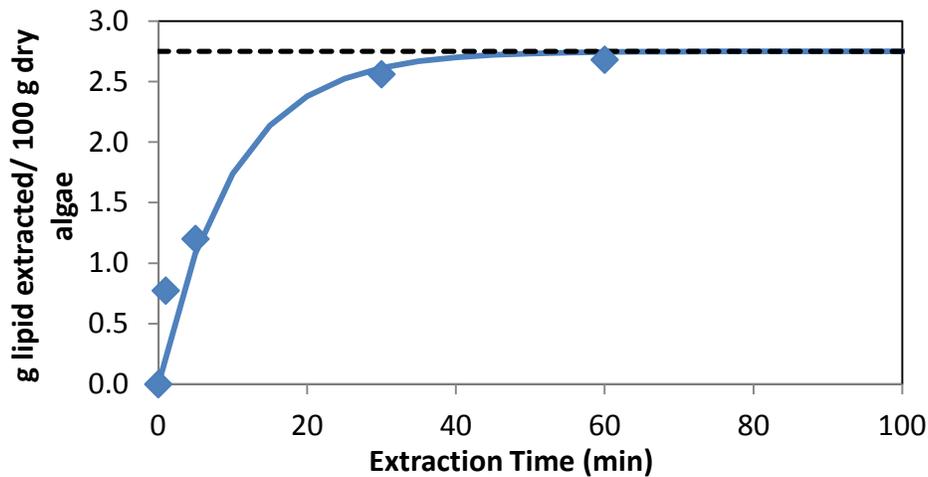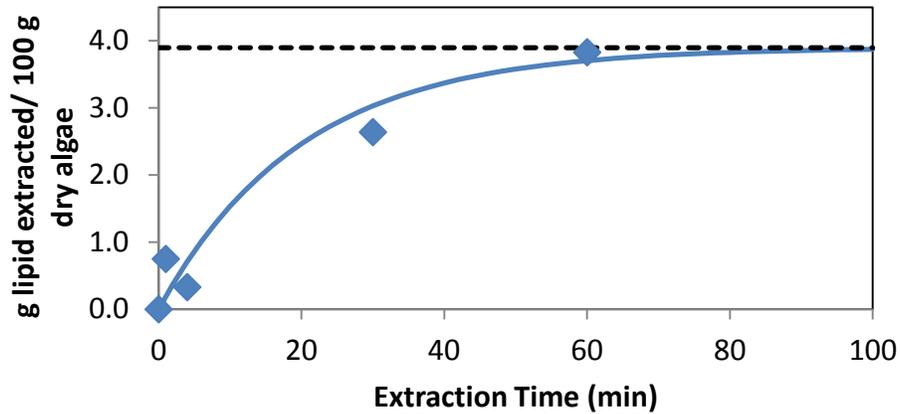
Figure 5.4: First Order Extraction Curve for the Hexane solvent extraction of microalgal lipids at a solvent to algae ratio of 30:1.

Figure 5.5 shows the first order extraction curve for the heptane solvent extraction of microalgal lipids at a solvent to algae ratio of 5:1. The rate of extraction is very quick. The extraction has basically gone to completion after 4 minutes. The maximum yield is 1.80 g lipid extracted per 100 g of dry algae. At four minutes, the yield is 1.64 g lipid extracted per 100 g of dry algae so the extraction is already 91% complete. The extraction does not need to be conducted for longer than 7 minutes because at that point, the extraction has gone to completion. The yield at the 60 minute mark is slightly lower than the yield at 4 and 30 minutes; this is due to experimental error. The data for this extraction (heptane at 5:1) fits the model well. The time constant was determined to be 2 minutes.

Figure 5.5: First Order Extraction Curve for the Heptane solvent extraction of microalgal lipids at a solvent to algae ratio of 5:1.

Figure 5.6 shows the first order extraction curve for the heptane solvent extraction of microalgal lipids at a solvent to algae ratio of 30:1. The rate of extraction is very quick at the beginning of the process, and over time it slows. At 4.75 minutes, the yield is 1.1 g lipid extracted per 100 g of dry algae. The maximum yield is 2.61 g lipid extracted per 100 g of dry algae so at the four minute mark, the extraction is already 42% complete. At 30 minutes the yield is 2.60 g lipid extracted per 100 g of dry algae which is basically the maximum. At 60 minutes, the extraction has gone to completion. Performing the extraction for longer than 60 minutes

probably does not make economic sense. The energy required to continue the extraction probably exceeds the energy contained in the extra lipids which could be extracted. The data for this extraction (heptane at 30:1) fits the model well. The time constant was determined to be 7 minutes.

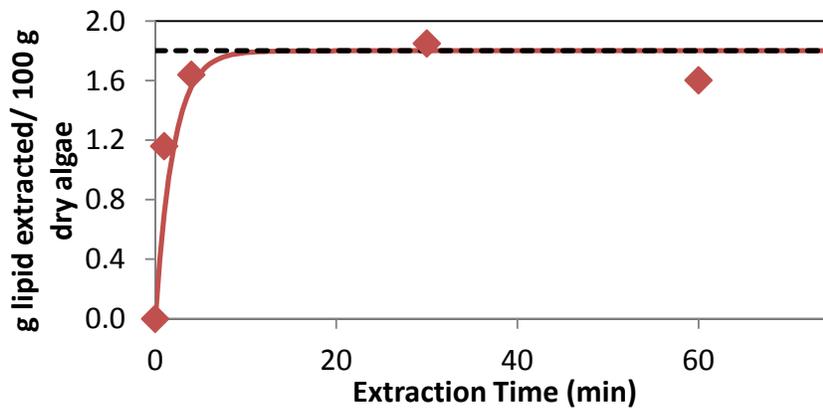Figure 5.6: First Order Extraction Curve for the Heptane solvent extraction of microalgal lipids at a solvent to algae ratio of 30:1.

5.4 Effect of the solvent to dry algae ratio (R) (Objective 7)

Figure 5.7 shows the first order extraction curves for the hexane solvent extractions of microalgal lipids at solvent to dry algae ratios of 5:1 and 30:1. For extractions less than 20 minutes, solvent to algae ratios of 5:1 and 30:1 will produce similar results. The 5:1 extraction is basically at completion at 20 minutes; after 20 minutes, the curve levels off. On the other hand, the 30:1 extraction is not done at 20 minutes. After 20 minutes, the amount of lipids extracted continues to increase. If hexane lipid extraction is to be done for less than 20 minutes, it is recommended to use a solvent to algae ratio of 5:1 since this will minimize solvent expenses without sacrificing results. If the hexane oil extraction is to be done for longer than 20 minutes, it is recommended to use a solvent to algae ratio of 30:1.

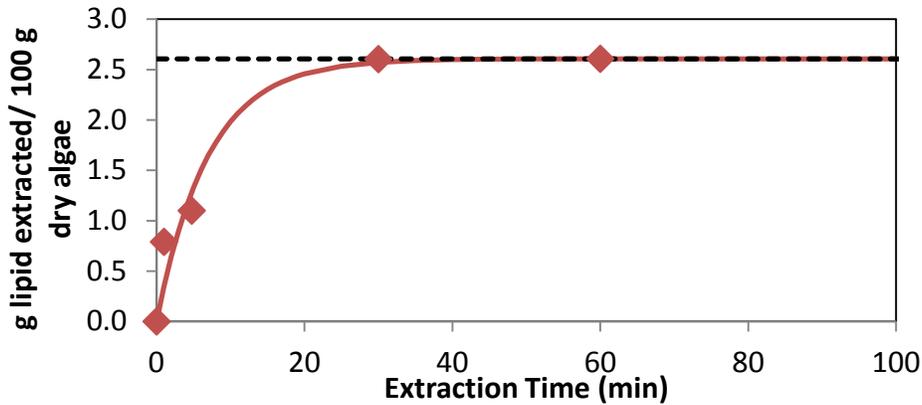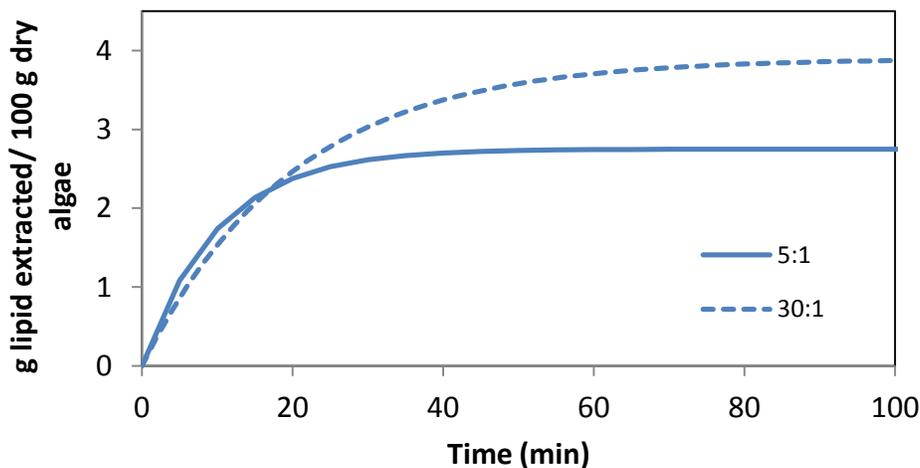Figure 5.7: Comparison of Extraction Kinetics for Hexane at solvent to algae ratios of 5:1 and 30:1.

Figure 5.8 shows the first order extraction curves for the heptane solvent extractions of microalgal lipids at solvent to algae ratios of 5:1 and 30:1. For extractions with heptane which last less than 10 minutes, a solvent to algae ratio of 5:1 is recommended because it will give a higher extraction yield. For extractions longer than 10 minutes, it is recommended that a solvent to algae ratio of 30:1 be used because the yield will be higher.
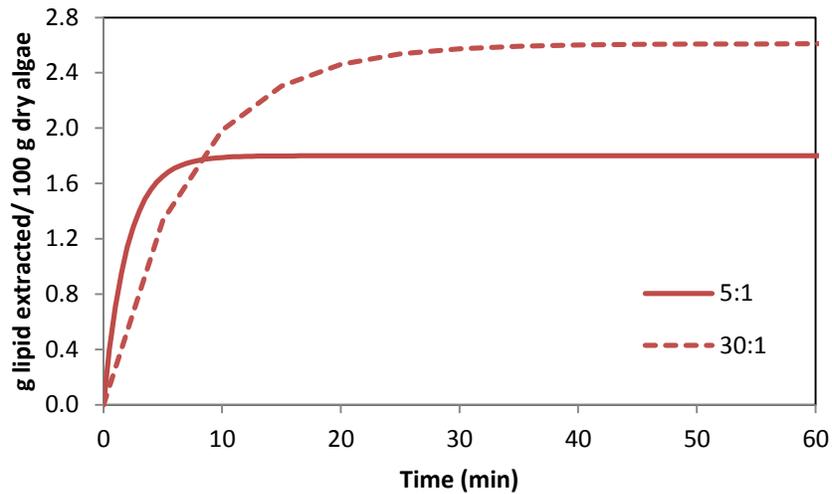
Figure 5.8: Comparison of Extraction Kinetics for Heptane at solvent to algae ratios of 5:1 and 30:1.

After comparing Figs. 5.3-5.6, it was determined that the hexane extraction at a 5:1 solvent to algae ratio had very similar kinetics to the heptane extraction with a 30:1 solvent to algae ratio. For hexane at 5:1, the maximum yield was 2.75 g lipid extracted per 100 g of dry algae whereas the maximum yield for the heptane at a 30:1 ratio was 2.61 g lipid extracted per 100 g of dry algae. The time constant for the hexane at 5:1 extraction was 10 minutes, and the time constant for the heptane at 30:1 was 7 minutes. This shows that to get similar results with hexane and heptane, the heptane volume must be six times larger than the hexane volume. Figure 5.9 shows the similar hexane and heptane extraction curves.
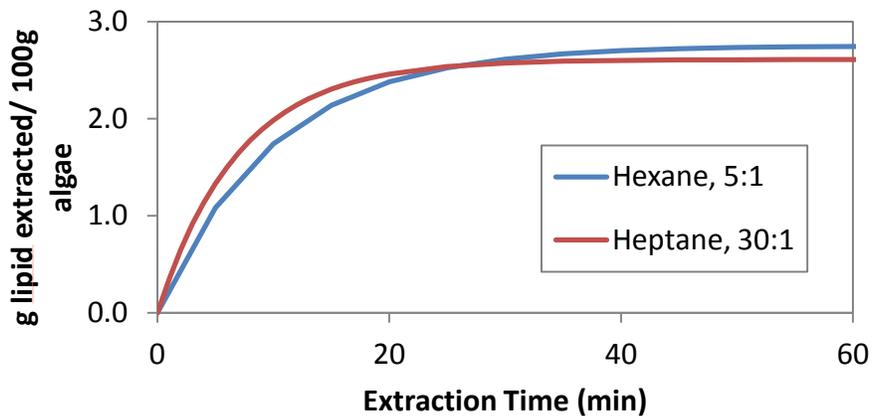
Figure 5.9: Comparison of the Extraction Kinetics for Hexane at 5:1 and Heptane at 30:1.

Table 5.2 gives a summary of the kinetics parameters for the four types of extractions conducted in this project. Looking at hexane, increasing the solvent volume by a factor of 6, doubled the time constant from 10 min to 20 min. Looking at heptane, increasing the solvent volume by a factor of 6, increased the time constant by a factor of 3.5 (from 2 min to 7 min). At a solvent to algae ratio of 5:1, the time constant for hexane is five time larger than the time constant for heptane. At a solvent to algae ratio of 30:1, the time constant for hexane is slightly less than three times the time constant for heptane. For hexane, increasing the solvent volume by a factor of 6, increased the maximum yield by 42% (2.75 to 3.90 g lipid extracted/100 g dry algae). For heptane, increasing the solvent volume by a factor of 6, increased the maximum yield by 45% (1.80 to 2.61 g lipid extracted/100 g dry algae). At a solvent to algae ratio of 5:1, hexane's maximum yield was 53% larger than heptane's maximum yield (2.75 vs. 1.80 g lipid extracted/100 g dry algae). For a solvent to algae ratio of 30:1, hexane's maximum yield was 49% larger than heptane's maximum yield (3.90 vs. 2.61 g lipid extracted/100 g dry algae). Figure 5.10 shows the extraction curves for hexane and heptane at a solvent to dry algae ratio of 5:1. Figure 5.11 shows the extraction curves for hexane and heptane at a solvent to dry algae ratio of 30:1.

Table 5.2: Summary of Kinetic Parameters

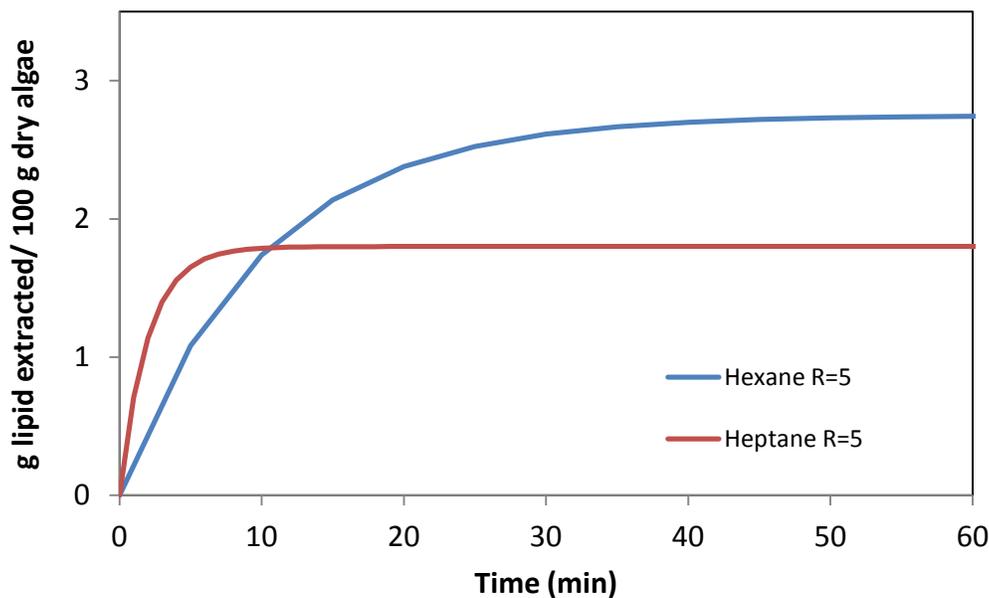|	Hexane 5:1	Hexane 30:1	Heptane 5:1	Heptane 30:1
Time Constant τ, min	10	20	2	7
Maximum Extractable Lipid, K, g/100 g algae	2.75	3.90	1.80	2.61

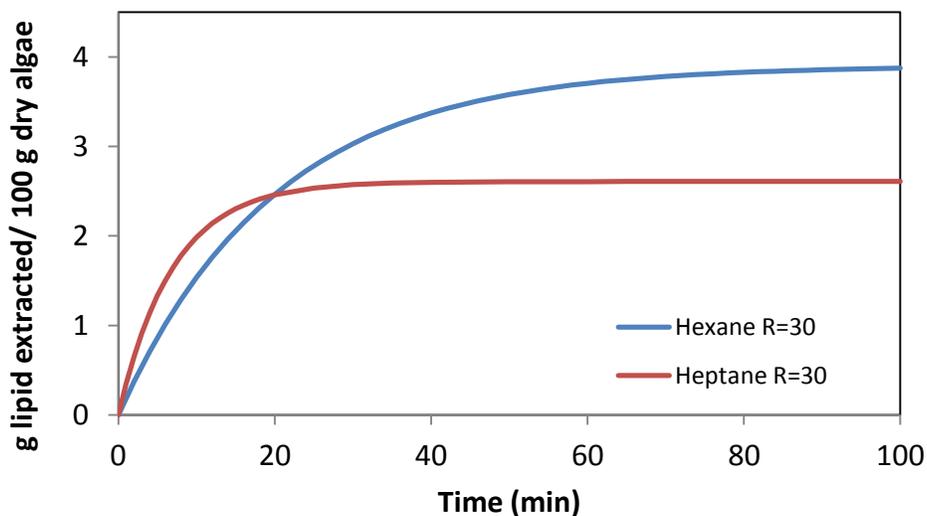Figure 5.10: Comparison of the extraction curves for hexane and heptane at R = 5.

Figure 5:11: Comparison of the extraction curves for hexane and heptane at R = 30.

5.5 Algae Oil Quality

It is desirable to evaluate the quality of the extracted oil, i.e., color, viscosity, clarity, and others. The experiments run in the laboratory did not generate enough microalgae oil to check the quality or compare the quality of hexane-extracted and heptane-extracted algae oil. A pilot scale photobioreactor with a volume of at least 500 L is needed. This should generate enough oil for quality and additional testing.

5.6 Comparison to Literature (Objective 8)

There is only a small amount of literature covering the kinetics of the lipid extraction from microalgae. Many studies only use lipid extractions to determine the amount of lipid in the algae they have grown. They usually do not consider the economics of the extraction process. Fajardo et al. (2007) analyzed the kinetics of the lipid extraction from microalgae using ethanol. It is difficult to compare kinetic parameters with this study because the solvents are different. Ethanol has both polar and non-polar properties so it extracts polar and non-polar lipids. This means that more material will be extracted by ethanol (not all of the extracted material can be converted to biodiesel though) than hexane or heptane. However, the extraction curves reported by Fajardo et al. (2007) were also first order processes (same shape as the curves obtained in this project). This means that the extraction itself is first order; the solvent does not determine the order of the extraction. Halim et al. (2012) summarized the extraction results from several other published articles. Unfortunately, all of the studies used co-solvents, both a polar and non-polar solvent paired together. This means that these extractions pulled out neutral and polar lipids. Again, this means that the yield for these extractions will be much higher than the yields obtained in this project. Most of the studies reported by Halim et al. ran their extractions for one hour which matches the longest extractions done in this project.

Table 5.3 and Figure 5.12 show the time constants for hexane lipid extractions with algae, canola seed, and olive cake. The time constant for the hexane lipid extraction from microalgae with a solvent to algae ratio of 5:1 and particle diameter of 2 to 10 μm is 10 minutes. The time constant for the hexane oil extraction from canola seed with a solvent to seed ratio of

4:1 and particle diameter of 60 μm is 28.6 minutes. The time constant for the hexane oil extraction from olive cake with a solvent to biomass ratio of 4:1 and particle diameter of 69 μm is 9.2 minutes. Comparing different biomasses, with different particle diameters and solvent to biomass ratios is very difficult, but it shows that the time constants are in a similar range.

Table 5.3: Comparison of Time Constants for Lipid Extraction using Hexane with different Biomass Sources.

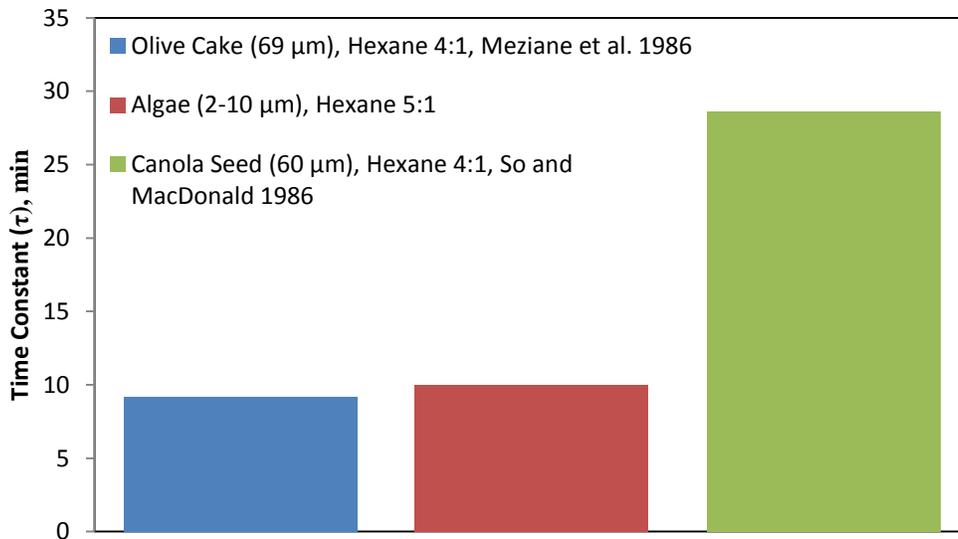|	Algae (2-10 μm) Hexane 5:1 This Work	Canola Seed (60 μm) Hexane 4:1 So and MacDonald 1986	Olive Cake (69 μm) Hexane 4:1 Meziane et al. 1986
Time Constant τ, min	10	28.6	9.2

Figure 5.12 Hexane Lipid Extraction Time Constant (minutes) for different Biomass Sources, olive cake, Chlorella vulgaris microalgae and canola seed.

CHAPTER 6

CONCLUSIONS

All the Project Objectives have been completed. The maximum yield (K) and time constant (τ) of the extraction for solvent-to-algae ratios of 5:1 and 30:1 for hexane and heptane were determined. The conclusions from the experiment results for the lipid extractions from Chlorella vulgaris are:

- Based on absorbance measurements, hexane and heptane extract very similar material.

- The lipid extraction from microalgae follows a first order process.

- Heptane extracts lipids faster than hexane. This is mostly likely because heptane boils at a higher temperature than hexane (both extractions were done at reflux). From mass transfer, the diffusion rate increases at higher temperatures.

- Hexane solvent extraction results in a higher maximum extracted lipids yield (K) than heptane.

- Increasing the solvent to algae ratio (R) from 5 to 30 (factor of 6), roughly doubled time constant for hexane (10 min to 20 min), increased the time constant for heptane by a factor of 3.5 (2 min to 7 min for heptane), increased hexane's K by 42% (2.75 to 3.90 g lipid extracted/100 g dry algae), and increased heptane's K by 45% (1.80 to 2.61 g lipid extracted/100 g dry algae).

- For an R of 5, hexane's time constant τ was five times that of heptane (10 min for hexane and 2 min for heptane). For an R of 30, hexane's τ was just under a factor of 3 larger than heptane's τ (20 min for hexane and 7 min for heptane). For R = 5 hexane's K was 53% larger than heptane's K (2.75 vs. 1.80 g lipid extracted/100 g dry algae). For R = 30, hexane's K was 49% larger than heptane's K (3.90 vs. 2.61 g lipid extracted/100 g dry algae).

- To get similar extraction results (speed and yield of extracted lipid), the heptane volume must be six times greater than the volume of hexane.

There were no published results on the kinetics of the lipid extraction from Chlorella vulgaris using hexane or heptane. Limited hexane extraction data showed that the time constant for algae oil is comparable to olive cake and canola seeds.

CHAPTER 7

RECOMMENDATIONS FOR FUTURE WORK

The following investigations need to be studied in detail in order to address questions or some difficulties encountered by the present work. These difficulties/questions are important to further understand the mechanism of lipids transfer from the algae to the solvent phase. However, most of these questions and/or difficulties were not among the main goals of the present work.

1- It is recommended that future researchers test other solvent to algae ratios (R) so the kinetics parameters, the maximum yield and time constant, can be expressed as a function of the solvent to algae ratio ($K = f(R)$ and $\tau = f(R)$). Once these functions have been determined, they can be used to economically optimize the lipid extraction process. If the extraction time is too short, not enough lipids will be extracted, but if the extraction is too long, it may not be cost effective to continue heating the system. There is a balance between money and energy invested and the money and energy gained.

2- Determine the quality of the extracted lipids (color, odor, viscosity, etc.). This requires using a pilot-scale photobioreactor with a volume of at least 500 L to produce enough lipids for testing.

3- Since most industrial applications of oil extraction are continuous, it would be desirable to design and construct a continuously operated lipids extraction unit to test the applicability of the first order model and kinetics parameters derived in the present work.

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APPENDIX A

NUTRIENT SOLUTION COMPOSITION

Nutrient Solution Composition			
Name	**Chemical Formula**	**Molecular weight (g/mol)**	**Concentration**
Calcium Chloride	$CaCl_2$	110.98	0.2 mM
Boric Acid	H_3BO_3	61.83	0.13 mM
Potassium Nitrate	KNO_3	101.10	5.2 mM
Magnesium Sulfate	$MgSO_4$	104.36	5 mM
Sodium Phosphate	Na_2HPO_4	141.96	0.4 mM
Sodium Chloride	$NaCl$	58.44	0.1 M
EDTA	$C_{10}H_{16}N_2O_8$	292.24	26.9 mg/L
Ferrous Sulfate	$FeSO_4 \cdot 7H_2O$	278.02	2.8 mg/L
Zinc Sulfate	$ZnSO_4 \cdot 7H_2O$	287.55	0.288 mg/L
Molybdenum Trioxide	MoO_3	143.94	0.125 mg/L
Copper Sulfate	$CuSO_4 \cdot 5H_2O$	249.68	0.075 mg/L
Cobalt Chloride	$CoC_{l2} \cdot 6H_2O$	237.93	0.025 mg/L
Manganese Chloride	$MnC_{l2} \cdot 4H_2O$	197.90	0.15 mg/L

APPENDIX B

EQUIPMENT LIST

Equipment Used in Experimental Work		
Equipment	**Model**	**Description**
Spectrophotometer	Bausch & Lomb Spectronic 21	For measuring the turbidity of the algae solution over time
Microscope	AO Spencer	For performing cell counts of the algae solution over time
Counting chamber and cover slides		For performing cell counts
Scale	AccuLab Vicon	For massing nutrients, algae, filter paper, etc.
Centrifuge	Damon/IECB-20A	For harvesting the algae - separate the algae from the nutrient medium
Lyophilizer (freeze dryer)	Labconco Freeze Dry 5	For freeze drying to obtain dry algae biomass
Extraction apparatus		For extraction lipids from the dry algae biomass
Hotplate/Stirrer		For heating and stirring the hot water bath and round bottom flask during extractions

APPENDIX C

EXPERIMENTAL DATA

The following tables present the experimental data used to create Figures 5.3 to 5.9.

Hexane, R = 5 mL of solvent used per g dry algae	
Extraction Time. min	g lipid/100 g dry algae
0	0
1	0.773
5	1.2
30	2.56
60	2.68

Hexane, R = 30 mL of solvent used per g dry algae	
Extraction Time, min	g lipid/100 g dry algae
0	0
1	0.750
4	0.330
30	2.64
60	3.83

Heptane, R = 5 mL of solvent used per g dry algae	
Extraction Time, min	g lipid/100 g dry algae
0	0
1	1.16
5.5	1.68
30	1.80
60	1.60

Heptane, R = 30 mL of solvent used per g dry algae	
Extraction Time, min	g lipid/100 g dry algae
0	0
1	0.79
4.75	1.1
30	2.57
60	2.61