Kinetics Study of the Solvent Extraction of Lipids from Chlorella vulgaris

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Abstract*— The aim of this investigation was to reduce the chemical hazard and possibly the production time of biodiesel from microalgae by investigating the process of hexane lipid extraction. This extraction process is energy-intensive and time-consuming. Hexane, a toxic solvent, is traditionally used for microalgae lipid extractions. The goals of this project were to (1) evaluate the less hazardous heptane as a substitute for hexane and (2) determine the kinetics of the extraction process for hexane and heptane at different solvent volume to dry microalgae mass ratios (R, mL solvent/g dry microalgae). To accomplish the first goal, the project included growth, monitoring, and harvesting of Chlorella vulgaris microalgae in reverse osmosis water and analyzing the lipid extraction using hexane and heptane. The second goal was accomplished by proposing a kinetics model of lipid extraction, measuring the hexane and heptane extracted lipids as function of the extraction time and fitting the data to the kinetic model. Using a spectrophotometer, it was determined that hexane and heptane extract similar material. For both solvents, the extraction process followed a first order model: $\tau\, dY/dt = (K-Y)$, where Y is the grams of lipid extracted per 100 gram dry microalgae, K is the maximum gram of lipid which can be extracted in the process per 100 gram dry microalgae, t is the extraction time (min), and τ is the time constant for the process (min). A smaller τ implies a faster extraction process, while a higher K indicates a higher lipid extraction yield. It was found that for hexane with R = 5, K = 2.75 and τ = 10. Increasing R to 30 amplifies K to 3.90 and τ to 20. For heptane with R = 5, K = 1.80 and τ = 2. When R was increased to 30, K rose to 2.61 and τ to 7. For a given R, heptane extraction is faster, but hexane extraction has a higher maximum yield.

***Index Terms*— Biodiesel, Chlorella vulgaris, Extraction Kinetics, Heptane Extraction, Hexane Extraction, Lipid Extraction.**

A. Microalgae Lipid Composition

The United States Department of Energy studied the algae to biodiesel process starting in the 1970's [1]. Recently, the production and characterization of microalgae biodiesel has expanded [2-25] and spread to many countries [26]. Lipids and fatty acids exist in algae. They function as membrane components, storage products, and as energy source. Lipids are classified according to their polarity. Table I. summarizes the characteristics of polar and non-polar compounds

Microalgae have both non-polar (lipophilic or neutral) carbon chains (like fatty acids) and polar (hydrophilic) lipids. The major part of the non-polar (neutral) lipids are triglycerides and free fatty acids. Triglycerides-esters are the major components of oils. These consist of one molecule of glycerol and three molecules of fatty acids. Polar or hydrophilic lipids, like phospholipids are glycerides in which a polar group replaced one or more of the fatty acids. The non-polar triglycerides are used to create biodiesel by the transesterification reaction. Hence, their efficient extraction is very important in the production of microalgae biodiesel. The characteristics of an oil are usually described in terms of its fatty acids composition. Fatty acids have a carboxyl group (COOH) which is polar and a hydrocarbon chain which is non-polar. The number of carbon atoms and double bonds in the hydrocarbon chain describes fatty acids. The most common fatty acids in edible oils have 18 carbon atoms.

Table I. Features of Polar and Non-Polar Compounds

Polar Compounds	Non-Polar Chemicals
Covalent compounds with the shared pair of electrons moves towards the atom with greater mass involved in the bonding	Covalent compounds with the shared pair of electrons are at an equal distance from the two bonded atoms
The greater mass atom will have a slight negative charge. The other atom will have a slight positive charge. The two centers of charge cause the molecule to be "dipole"	There is no separation in the positive and negative charges of the molecule
Higher melting and boiling points compared to non-polar compounds	Relatively lower melting and boiling points
Relatively stronger forces between molecules	Weak Van der Waal's forces between molecules
Polar solutes dissolve in polar solvents	Non-polar solutes dissolve in non-polar solvents
Solvate and form ions when dissolve in water	Not water-soluble. Hence these do not dissociate to form ions in water
Conduct electricity in aqueous solution	Do not conduct electricity in aqueous solution
Examples include: water, urea, methanol, ethanol, formic acid	Examples include: pentane, toluene, hexane, heptane, benzene

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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 [18, 27]. The most common fatty acid in *Chlorella vulgaris* is the unsaturated Linoleic acid, with two double bonds (18:2) [28], Fig. 1.

B. *Microalgae Lipid Extraction*

Halim et al. [27] proposed the following five-step mechanism for the solvent lipid extraction from microalgae.

1. The solvent penetrates the external surface of the cell, i.e., 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 solvent-lipids complex moves through the static film surrounding the cell (boundary layer) and enters the bulk solvent.

Figure 2 shows the five-step lipid extraction mechanism.

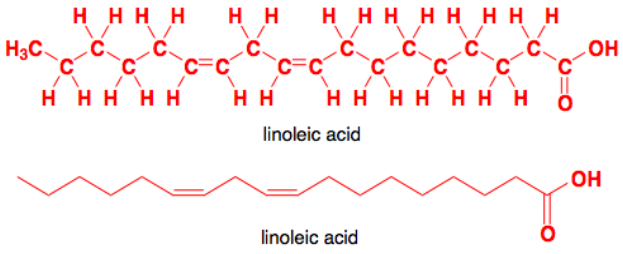

Fig. 1. Linoleic Acid Structure. Reproduced from http://www.chemtube3d.com/ClaydenLinoleic.html

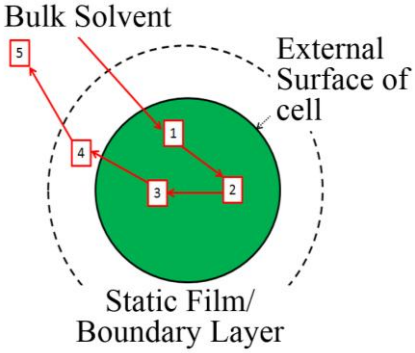

Fig. 2. Lipid Extraction Mechanism.

C. *Hexane Solvent Substitution*

Hexane is a hydrocarbon with the chemical formula $CH_3(CH_2)_4CH_3$ or simply C_6H_{14}. The structures of n-hexane and n-heptane are shown in Fig. 3.

n-Hexane 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 II.

Ayers and Dooley [29] 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.

Pons and Eaves [30] compared four 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 alternatives. Taha *et al.* [31] who studied the solvent extraction of oil from cottonseed further confirmed this.

MacGee [32] 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 [29] 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.* [33] noted that heptane extracted more phospholipids than hexane. Conkerton *et al.* [34] 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

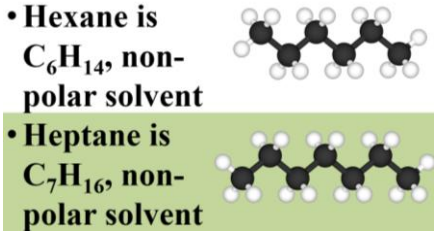

Fig. 3. N-Hexane and n-heptane structures

Table II: 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

extraction. This was in agreement with earlier results of Johnson and Lusas [35]. They concluded that heptane offers a potential alternative to hexane for oil extraction from cottonseed.

D. Extraction Kinetics Modeling

Patricelli et al. [36] 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:

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

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

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

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 of this study is:

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

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, many 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.

II. Kinetics Modeling of Lipid Extraction from Dry Microalgae

Figure 4 shows a schematic of the extraction of lipids from dry lipid-rich microalgae using hexane solvent.

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

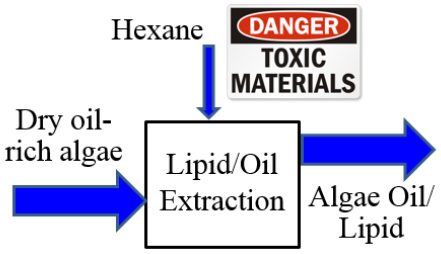

Fig. 4. Schematic of Lipid Extraction from Dry Lipid-Rich Microalgae.

A. Derivation of Mathematical Model

The mass transfer of lipids during solvent extraction can be described by a first order model [37]. The kinetics model equation is

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

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 [27]. Solving the differential equation gives:

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

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 the Patricelli et al. [36] model without a washing stage.

B. Mathematical Model and Physical Situation

The model matches the physical situation. Figure 5 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.

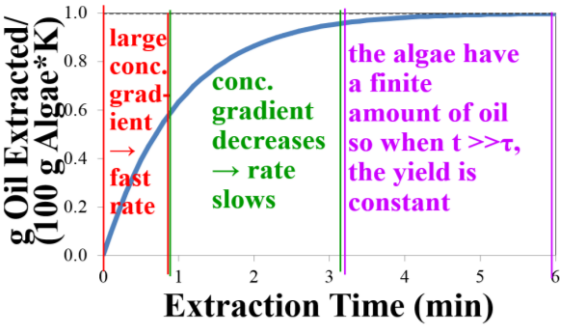

Fig. 5: *Example Plot of the Microalgae Lipid Extraction Model.*

C. Relating the Kinetic Parameters to Experimental Data

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

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

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

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

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

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

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 zero. The derivative of the SSE with respect to K is

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

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

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

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

$$\frac{\partial SSE}{\partial\left(\frac{1}{\tau}\right)} = \sum_{i=1}^{N} 2\left(Y_i K + K e^{-t_i/\tau}\right)\left(-K t_i e^{-t_i/\tau}\right). \quad (9)$$

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

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

D. Relating the Kinetic Parameters to Experimental Data

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, K.

III. Project Purpose and Goals

A. Project Purpose

Many investigators have analyzed microalgae growth, extraction of microalgae lipids, and biodiesel production from the extracted microalgae lipids. However, commercial scale production of microalgae lipids/oil is still not cost-effective. Considerable research is taking place to trigger lipid formation in microalgae [4, 8], and reduce the energy and water requirements for growing microalgae [9, 19]. The extraction of lipids from lipid-rich microalgae has the following characteristics:

- Hexane is very toxic to the peripheral nervous system
- Solvent extraction is energy-intensive and time-consuming (thus costly)

It is highly desirable to find a substitute solvent for the extraction of lipids from microalgae that would lower the safety concern issues and reduce the extraction time.

B. Project Goals

The goals of this project were to:

- Select a less toxic solvent for the process of lipid extraction from microalgae.
- Determine the maximum amount of lipids which can be extracted in the process per 100 gram of dry microalgae (K) and the time constant for the process (τ) for different solvent volume to dry microalgae mass ratios (R, mL solvent/g dry microalgae) using hexane and an alternative solvent.

IV. Materials and Methods

A. Microalgae Species and Growth Requirements

Chlorella vulgaris was grown in a nutrient medium containing macronutrients and micro-nutrients. *Chlorella vulgaris* is a green alga which is very resilient; it can be grown in wastewater [3,12,16,18,19]. Reverse Osmosis (RO) water was used to create the nutrient mediums in this project. 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.

B. Microalgae Harvesting

A Damon/IEC B-20A centrifuge was used to remove the majority of the water from the microalgae solution. The medium spun in the centrifuge at 5000 rpm for 10 minutes. Then, the samples were freeze dried at -80°C under vacuum for 48 hours using a Labconco Freeze Dryer. At the end of the freeze-drying process, the microalgae 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 mixed in one container to obtain one homogenous mixture. Algae samples were taken from this mixture for the lipid extraction experiments.

C. Lipid Extraction Experimental Setup

The lipid extractions in this project followed the work of Ferrentino [15]. He used dry algae powder and a Soxhlet extractor. He 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, the hexane was evaporated to obtain dry lipids. The lipid content was reported as the mass of 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. This reduced the risk of breaking glassware or other accidents. Figure 6 shows the setup.

D. Lipid Extraction Procedure

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 microalgae in this project were ground to a fine powder. The majority of extractions completed for this project were done with 5.00 g of microalgae. This amount of algae was large enough so that there were no inaccuracies with mass measurements, but not so large that the algae supply was used up quickly. The solvent (mL) to algae (g) ratio R determined the amount of solvent to use once the algae had been weighed out. For this project, R values 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.

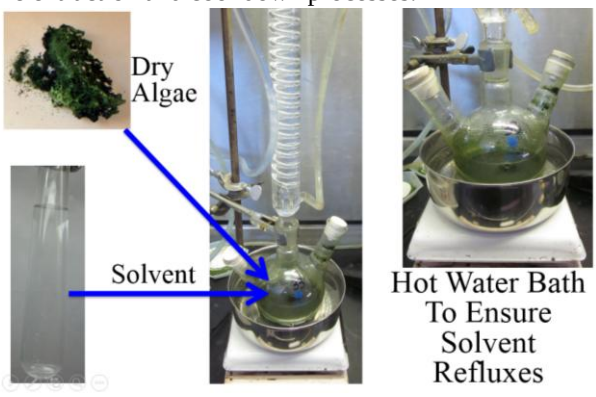

Fig 6: Experimental Setup for the Extraction of Lipids from dry Microalgae using either Hexane or Heptane solvent

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, as shown in Fig. 7, the solvent was evaporated which left the dry solvent-free lipids in the flask. The mass of lipids recovered was determined and used to calculate the extraction yield, Y.

E. Measurements and Metrics

The measured variables and purpose/metrics calculated are given in Table III.

V. Results/Discussion/Accomplishments

A. Solvent Selection

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 IV shows the physical properties for hexane and heptane.

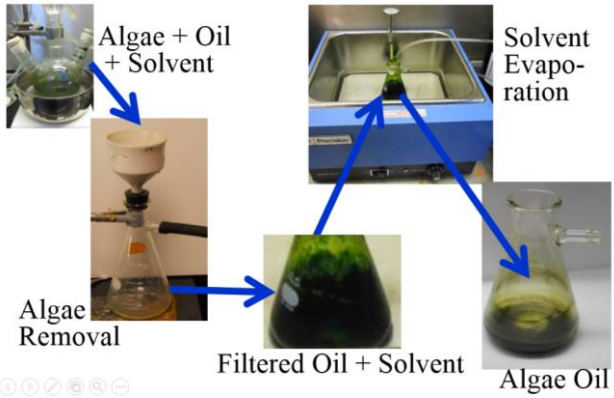

Fig 7: Experimental Setup for the removal of algae and recovery of extracted algae oil from the solvent.

Table III. Measured Variables and Metrics Calculated.

Measurement	Purpose/Metrics
Daily microalgae solution turbidity (using spectrophotometer)	Microalgae growth monitoring to determine when to harvest microalgae
Daily cell counts of microalgae in solution (using microscope and hemocytometer)	Microalgae growth monitoring to determine when to harvest microalgae
Microalgae mass after harvesting and drying (using balance)	Determine microalgae production and track amount of microalgae available for extractions
Length of solvent extraction process (using stopwatch)	Extraction time is needed to determine process kinetics
Mass of microalgae lipids extracted (using balance)	Determine extraction yield (Y)
Absorptivity of extracted lipids in solvent (using spectrophotometer)	Determine if hexane and alternative solvent extract similar material

Table IV: Properties of Hexane and Heptane [38]

Property	Hexane	Heptane
Chemical Formula/ Structure	C_6H_{14}	C_7H_{16}
Solvent type	Non-polar	Non-polar
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

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.

B. Heptane Viability

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. There was a major absorbance peak at 410 nm, and a minor absorbance peak at 640 nm. The procedure was repeated with heptane. Heptane had a major absorbance peak at 410 nm and a minor absorbance peak at 660 nm. Figure 8 shows the measured absorbances (scaled from 0 to 1) vs wavelength plot for mixtures of hexane/heptane and microalgae lipids. The 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.

C. Algae Oil Extraction Kinetics Model Parameters

Figure 9 shows the first order extraction curve for the hexane solvent extraction of microalgae lipids at a solvent to algae ratio R 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 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.

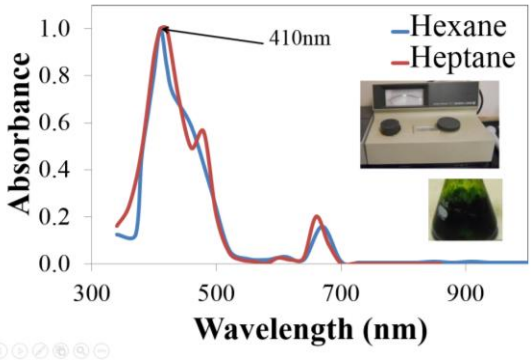

Fig. 8: Spectrophotometer-measured absorbance of extracted microalgae lipids and solvent (Hexane or Heptane) mixture versus Wavelength.

Figure 10 shows the first order extraction curve for the hexane solvent extraction of microalgal lipids at a solvent to algae ratio R 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.

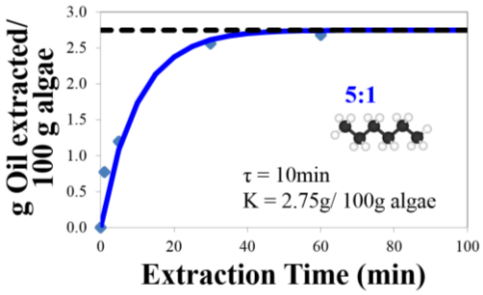

*Fig. 9: First Order Extraction Curve for the Hexane solvent extraction of microalgal lipids at a solvent to algae ratio of 5:1. The computed values of the τ (minutes) and K (*g lipid extracted per 100 g of dry algae *are shown.*

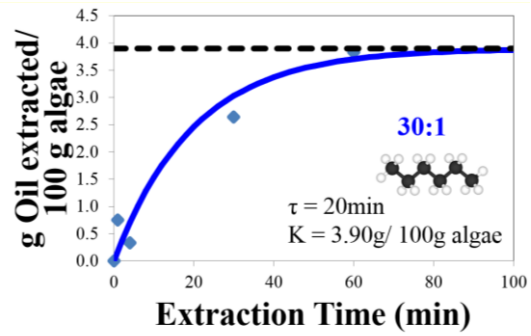

Fig. 10: First Order Extraction Curve for the Hexane solvent extraction of microalgal lipids at a solvent to algae ratio R of 30:1. The computed values of the τ and K are shown.

Figure 11 shows the first order extraction curve for the heptane solvent extraction of microalgal lipids at a solvent to algae ratio R of 5:1. The rate of extraction is very quick. The extraction has gone to completion after 4 minutes. The maximum yield K 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 3 minutes.

Figure 12 shows the first order extraction curve for the heptane solvent extraction of microalgal lipids at a solvent to algae ratio R 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 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 that 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.

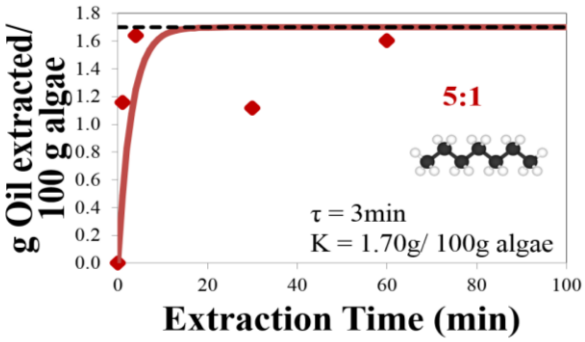

Fig. 11: First Order Extraction Curve for the Heptane solvent extraction of microalgal lipids at a solvent to algae ratio R of 5:1. The computed values of the τ and K are shown.

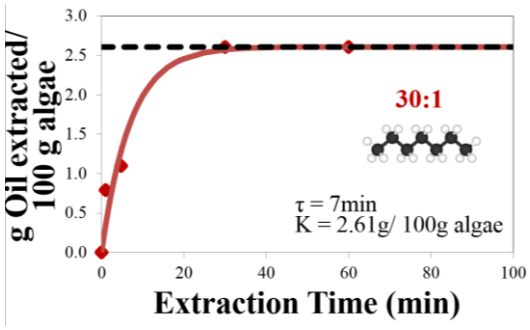

Fig. 12: First Order Extraction Curve for the Heptane solvent extraction of microalgal lipids at a solvent to algae ratio R of 30:1. The computed values of the τ and K are shown

D. Effect of the solvent to dry algae ratio (R)

Figure 13 shows the first order extraction curves for the hexane solvent extractions of microalgal lipids at solvent to dry algae ratios R 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 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 an R 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 an R of 30:1.

Figure 14 shows the extraction curves for the heptane solvent extractions of microalgal lipids at R of 5:1 and 30:1.

For extractions with heptane that last less than 10 minutes, an R of 5:1 is recommended because it will give a higher extraction yield. For heptane extractions longer than 10 minutes, it is recommended that an R of 30:1 be used because the yield will be higher.

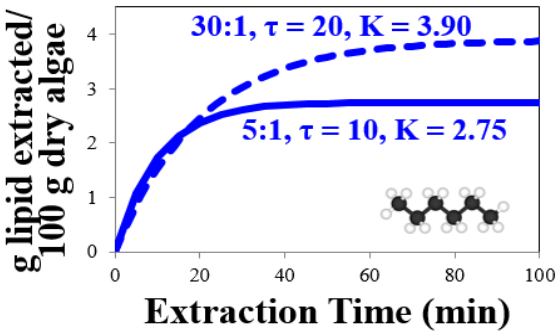

Fig. 13: Comparison of Extraction Kinetics for Hexane at solvent to algae ratios R of 5:1 and 30:1

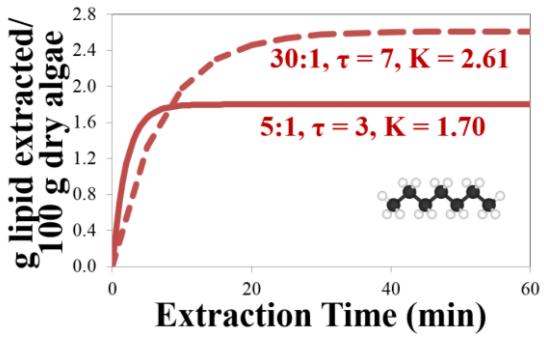

Fig. 14: Comparison of Extraction Kinetics for Heptane at solvent to algae ratios R of 5:1 and 30:1

Analysis of the results indicated that the hexane extraction at an R of 5:1 had very similar kinetics to the heptane extraction with an R of 30:1. For hexane at R= 5:1, the maximum yield K was 2.75 g lipid extracted per 100 g of dry algae whereas K for the heptane at an R of 30:1 was 2.61 g lipid extracted per 100 g of dry algae. The time constant τ for the hexane at R = 5:1 extraction was 10 minutes, and τ for the heptane at R= 30:1 was 7 minutes. This shows that similar extraction results are obtained when the heptane volume is six times larger than the hexane volume. Figure 15 shows the similar hexane and heptane extraction curves.

Table V 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 an R of 5:1, the time constant for hexane is five time larger than the time constant for heptane. At an R 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 six 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 six increased the maximum yield by 45% (1.80 to 2.61 g lipid extracted/100 g dry algae). At an R 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 an R 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). Figures 16 and 17 show the extraction curves for hexane and heptane at solvent to dry algae ratios, R of 5:1 and 30:1, respectively.

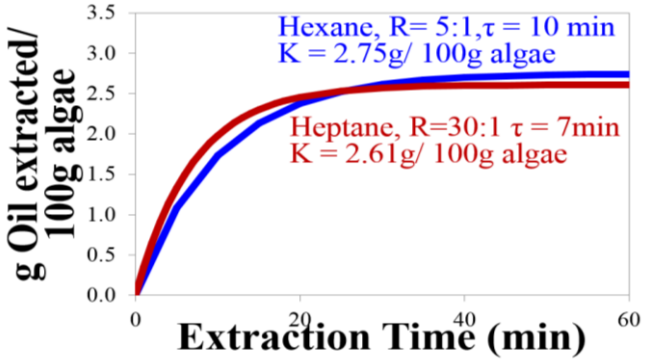

Fig. 15: Comparison of the Extraction Kinetics for Hexane at R = 5:1 and Heptane at R = 30:1

Table V: Summary of Kinetic Parameters

	Hexane R = 5:1	Hexane R = 30:1	Heptane R = 5:1	Heptane R = 30:1
τ, minutes	10	20	2	7
K, g lipid extracted per 100 g of dry algae	2.75	3.90	1.80	2.61

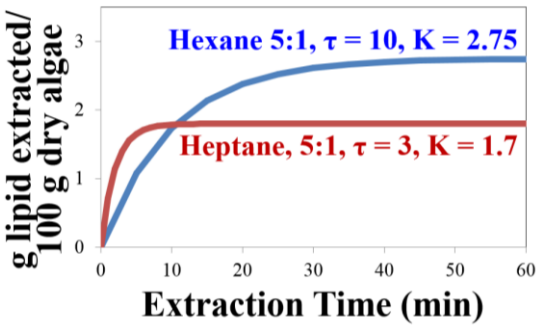

Fig. 16: Comparison of the extraction curves for hexane and heptane at R = 5

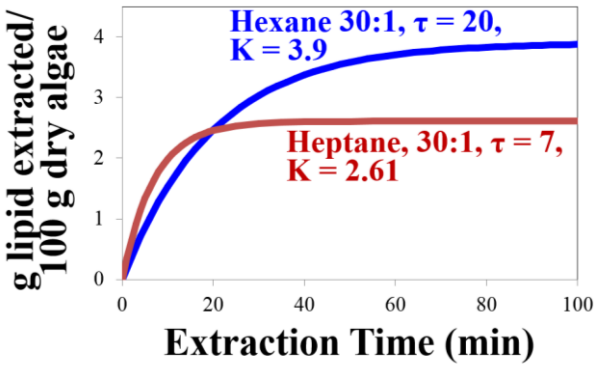

Fig. 17: Comparison of the extraction curves for hexane and heptane at R = 30

E. Comparison to Literature

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.* [39] 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.* [39] 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.* [27] 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.* [27] ran their extractions for one hour, which matches the longest extractions done in this project.

Table VI and Figure 18 show the time constants for hexane lipid extractions with algae, canola seed, and olive cake. 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 VI: Comparison of Time Constants (minutes) for Lipid Extraction using Hexane with different Biomass Sources

	Algae (2-10 μm) Hexane R = 5:1 This Work	Canola Seed (60 μm) Hexane R = 4:1 [40]	Olive Cake (69 μm) Hexane R = 4:1 [41]
Time Constant τ, minutes	10	28.6	9.2

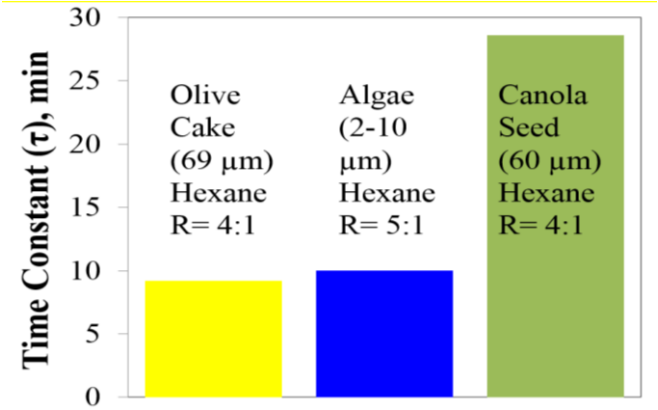

Fig. 18 Hexane Lipid Extraction Time Constant (minutes) for different Biomass Sources, olive cake, Chlorella vulgaris microalgae and canola seed

VI. Conclusion

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 most likely because heptane boils at a higher temperature than hexane. Higher temperature increase mass transfer rate.
- 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 obtain similar extraction results (speed and yield of extracted lipid), the heptane volume must be six times greater than the volume of hexane.

Acknowledgment

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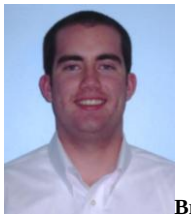

Brian McConnell graduated from the University of New Hampshire in May 2013 with a B.S. in Chemical Engineering (Summa Cum Laude) with University Honors. He completed several projects during his 3.5 years with the UNH Biodiesel Research Group. Some of his awards include: 2010 UNH Research Experience and Apprenticeship Program (REAP) grant, 2011 UNH Summer Undergraduate Research Fellowship (SURF), 2011 United States Environmental Protection Agency Greater Research Opportunities Fellowship for Undergraduate Environmental Study, 2012 Barry M. Goldwater Scholar, 2013 UNH Undergraduate Research Award, 2012 & 2013 UNH Undergraduate Research Conference Award of Excellence. He has co-authored two peer-reviewed journal articles and given presentations at two professional and four undergraduate research conferences.

Ihab H. Farag Professor Emeritus, Chemical Engineering at University of New Hampshire. In Sept 1976, he joined the faculty at UNH after receiving his doctorate of Science (Sc.D.) from the Massachusetts Institute of Technology (MIT) in Chemical Engineering. His research has focused on biofuels, microalgae biodiesel (renewable alternative to petro-diesel liquid fuel), bio-jet fuels, cleaner production, energy efficiency, and chemical risk screening applications. He initiated the Bio-Oil Group, the Biodiesel Research Group and the New Hampshire Pollution Prevention Internship Program to involve and mentor interdisciplinary students in research and industrial projects. Invited presentations and briefings were given to the legislators on Pollution Prevention, Biomass, Biodiesel and Bio-Oil. He has collaborated with several countries, e.g., Bulgaria, Cambodia, Egypt, El Salvador, Pakistan, Qatar, Thailand and Vietnam on the production of Biodiesel fuel from Jatropha and microalgae and on Pollution Prevention applications. These resulted in over 130 publications and presentations and being invited to be a Keynote speaker and to organize international and regional conferences.

Dr. Farag received a number of prestigious awards, e.g., the US EPA Environmental Merit award, the Coast Guard Meritorious Commendation, the US Most Valuable Pollution Prevention (MVP2) Program award, and the UNH award for Excellence in International Engagement. In addition, he received several Outstanding Teaching awards at MIT and UNH.