THESIS

EXTRACT CHARACTERISTICS OF SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF NANNOCHLOROPSIS OCULATA

Submitted by

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ABSTRACT

EXTRACT CHARACTERISTICS OF SUPERCRITICAL CARBON DIOXIDE EXTRACTION FROM NANNOCHLOROPSIS OCULATA

This research progresses the understanding of super-critical carbon dioxide extraction of an algal sample composed of *Nannochloropsis oculata*. The apparatus used in the experiments was optimized in several iterations to find the best balance of quality of data collection and practicality of operation. Thin-layer chromatography (TLC), gas chromatography (GC), and gravimetric analytical techniques were applied to the algae extract. The results indicate that 400 ± 4.5 bar and 83 ± 9.7°C recovered the largest proportion of extract on a mass basis. TLC analysis revealed a polar spot for some experiments, but it was not possible to verify that the spot was from the extraction and not a contaminant. GC analysis verified the lipid profile of the extract was modified by different extraction conditions and over the course of the extraction. The results reveal that additional research is warranted with super-critical carbon dioxide extraction of algae.

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I. Introduction

Much of the modern world relies on the internal combustion engine. So many aspects of life can be attributed to the transportation of goods or persons. Traditionally, internal combustion engines have utilized petroleum based oil to operate. These oils are extracted from around the world and refined to the specific fuel formula that each engine requires. It is estimated that there are only about 45 years of petroleum oil left in the world [1]. Although the formation of petroleum products in the earth is regenerative, the rate at which humans are extracting them is greatly outpacing the rate of their formation. These disproportionate rates raise the problem of a consumptive path.

One solution to the depletion of these petroleum based fuels would be a truly renewable source of fuel, such as an advanced biofuel. Biofuels offer a great advantage in renewability but they also pose their own problems based on the source of the biomass. Biofuels from feedstock crops would cause competition between food and fuel as an end product [2]. Biofuels from non-feedstock crops cause a competition between the arable land being used for cultivation of food or fuel [2]. A biomass source that would not require arable land and can be grown with brackish water instead of the clean fresh water most crops require is algae [3]. Algae have the potential to be an incredible biofuel source based on the land requirements, growth rate, and lipid production rate [4].

In order to use algae as a source of renewable fuels it is beneficial to understand the general steps required to go from algal biomass to a useable fuel [5]. The first stage of biofuel production is growth. This is something that algae have been doing naturally for a very long time, and some would argue that algae is one of the oldest life forms on earth [2]. The second stage is cultivation or harvest; this stage usually incorporates dewatering for benefit of downstream processing [6]. The third stage is extraction of the lipids. This stage is a major hurdle for algae biofuels as there is no established industry standard for the technique or solvent choice. The problem with extraction is not limited to the lack of understanding of different techniques, fuel is not a high value product and a costly extraction process cannot be justified. . The extraction process needs to be simple and appropriate to compete with the current low cost of fuels. The fourth stage is refinement of the extracted compounds into a usable fuel. A compound with single or multiple fatty acid esters can be transesterified in methanol to produce fatty acid methyl esters (FAMEs) or biodiesel [7]. The transesterifiable lipids that are of interest to this research are glycerides [4, 8, 9], sterols [10, 11], and phospholipids [9, 12, 13].

A promising process for lipid extraction is supercritical carbon dioxide, $SCCO_2$ [14]. The term supercritical refers to the physical state of the carbon dioxide. Supercritical fluids are fluids at a higher temperature and pressure than the critical point, where distinct liquid and gas phases do not exist, as shown in Figure 1 [14]. In this state the vapor and liquid phases are indistinguishable, meaning that supercritical fluids are a single phase [14]. Supercritical fluids are unique in that the density is similar to a liquid while the diffusion and viscosity is between a gas and liquid [15]. Table 1 shows the relative values of these properties for the liquid, gas, and supercritical phases of carbon

dioxide. Note that the temperature is specified because there is a possibility for variation of physical properties for gases and liquids based on the temperature. Table 1 demonstrates that a supercritical fluid has the ability to have a higher mass transfer rate than a liquid solvent [15]. Carbon dioxide is chosen as a solvent, as opposed to an organic solvent, because it is nontoxic, nonflammable, noncorrosive, and has a low critical point [16]. The lack of adverse health effects from CO_2 is one of the strong advantages of using it as a solvent. Another advantage is the low critical point of 73.8 bar and 31.1°C [9]. This allows for the extraction of compounds that may be thermally sensitive [17]. Another advantage of CO_2 over an organic solvent is that CO_2 will readily evaporate from the extract at ambient conditions whereas an organic solvent would leave a residual amount behind at ambient conditions [14]. $SCCO_2$ is currently being used to decaffeinate coffee beans, extract aromas from spices, extract lipids, create porous polymers, and for cold sterilization of hydrogels [18-21]. The main focus of the research presented in this thesis is the use of $SCCO_2$ for lipid extraction with the intent of producing biofuels.

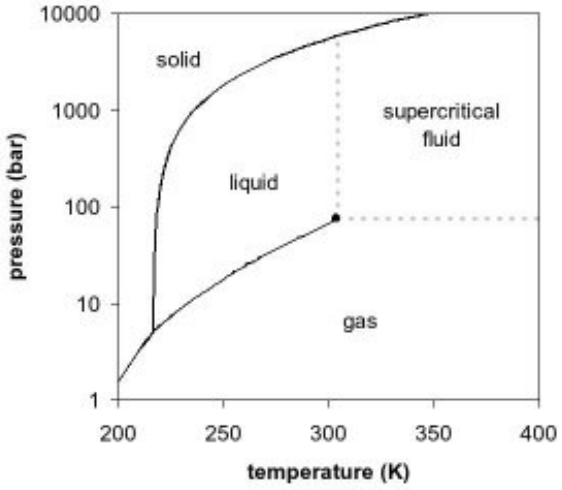

Figure 1. Phase diagram for carbon dioxide, illustrating the conditions where it becomes a supercritical fluid.

Table 1. Generalized physical properties for gas, liquid, and supercritical fluids [14].

State of fluid	Density (ρ, g/cm^3)	Diffusivity (D_{AB}, cm^2/s)	Viscosity (μ, g s/cm)
Gas $p = 1$ atm; $T = 21$ °C	10^{-3}	10^{-1}	10^{-4}
Liquid $p = 1$ atm; $T = 15$–30 °C	1	$<10^{-5}$	10^{-2}
Supercritical $p = p_c$; $T = T_c$	0.3–0.8	10^{-3}–10^{-4}	10^{-4}–10^{-3}

Survey of Literature

The research in the area of supercritical fluids began over 70 years ago [22], but the application of using supercritical carbon dioxide to extract lipids from algae is relatively recent [23]. With regards to the extract from $SCCO_2$ extraction, there is a desire to understand the process's outcomes quantitatively and qualitatively. The total mass and rate at which the extract is recovered is also important. Since the interest of this thesis is a biofuels approach, there is a desire to not only know which compounds are extracted but also the profile of the fatty acid chains as those affect the fuel quality after transesterification.

With any extraction research it is important to understand the properties of the starting material. This is especially true for the extraction of algae. The composition of the lipids in an algae cell will change throughout the life cycle based on external stresses [3, 4, 24-29]. These stresses can be temperature, amount of sunlight, or nutrient deprivation. The main source of stress that affects the lipid content is the supply of nitrogen being exhausted. If the supply of nitrogen is cutoff this will cause the algae to stop reproducing [25], and instead store excess carbon and energy as lipids, mostly

neutral lipids as triglycerides [4, 5, 27]. These triglycerides are one of the desirable transesterifiable compounds for biodiesel production.

Growth factors that affect the lipid composition of algae are not the only property to be concerned with in extraction research. Another important factor is how the algae were prepared for the extraction, which most researchers describe in detail. In order to effectively analyze an extraction, the water in the sample must be removed before extraction in order to isolate the effect of the solvent being used. Adverse outcomes are a possibility depending on the drying technique and must be considered. One such outcome is the possibility of hydrolysis when oven drying a wet sample. Hydrolysis occurs when there is water and heat present with a glyceride, the result is that a fatty acid chain is cleaved from the parent molecule to form a free fatty acid, an undesirable effect [30]. The preferred method for drying an algae sample is freeze-drying. This method sublimates water out of a frozen sample at low pressure. Freeze-drying provides a couple of benefits. The first is that it will create a very porous sample that has a high surface area to volume ratio, generally increasing extraction efficiency. The second benefit is that by removing water at a low temperature, hydrolysis of the glycerides can be avoided.

After preparing the sample, the next step in an extraction experiment is to conduct the study and analyze the results. There are a few main metrics used to compare the results of this study to the current $SCCO_2$ extraction literature. The first point is the total mass of extract and the extraction rate. The total mass extracted from a $SCCO_2$ extraction is easy to measure and compare to literature. However, the results reported in the literature strongly disagree with each other. Some of the researchers have found that the total mass of extract ends is the same for all test conditions [31-33]. Whereas many

other researchers have found the total mass varies based on the test conditions [16, 23, 34-43]. In order to reconcile these opposing results some researchers have shown that a long enough extraction duration results in the total mass of extract collected being the same for all test conditions [16, 36]. However other researchers have found that even with adequate extraction duration the total mass of extract is not the same for all test conditions [35, 37, 40, 43]. A single work attempted to bridge these opposing results, showing that for some of the more severe conditions, a similar extract mass was yielded whereas other less severe conditions did not yield the same mass [20]. Another researcher went as far as showing that at a specific pressure, 250 bar, the total mass of extract will be the same for all conditions [16]. However detailed the research, there doesn't exist an absolute agreement concerning the extraction mass for the works surveyed.

The total mass of extract collected from an extraction is dependent on the extraction rate. The extraction rate could be related to the solubility of the solutes in the $SCCO_2$. Some work has been presented arguing that the solubility of oil in $SCCO_2$ can vary with the conditions of the fluid. There is a common idea that the density of the CO_2 correlates to the solvating power [14, 35, 41, 44, 45]. However, this idea is not a complete explanation of $SCCO_2$ kinetics. It is believed that a pressure breakpoint exists. For pressures below this breakpoint, an increase in temperature would decrease the solubility of oils in $SCCO_2$. For pressures above this breakpoint, an increase in temperature would increase the solubility of oils in $SCCO_2$. The reason for this change comes from a change in the dominant factor of the extraction; from the density of the $SCCO_2$ dominating below the breakpoint to oil fugacity dominating above the breakpoint [44]. Many researchers agree to this idea of a breakpoint, but the exact pressure of this

breakpoint is not agreed upon. Andrich et al. proposed that the breakpoint is around 400 bar [44], Yu at al. states that the point is 350 bar [46], and Favati et al. suggests it is 300 bar [20, 36, 46]. In a more recent work by Marcías-Sánchez et al. 400bar is presented as being an accurate breakpoint for temperatures up to 50°C, but for temperatures above 50°C the pressure point may be only 300 bar [39]. Another factor that can greatly affect the extraction rate is the mass transfer resistance of cell walls [47]. This factor can be avoided by having the cell walls disrupted, but none of the literature surveyed stated there was a cell wall disruption step in the preparation of an algae sample. The overall factors that affect the extraction rate are a combination of the CO_2 properties, solute properties, and an interaction between those properties.

When all the factors affecting the extraction rate and solubility in $SCCO_2$ are examined as a whole, it is easy to understand that there are different kinetics in extraction for different solutes. Carbon dioxide is generally considered a nonpolar molecule, which makes it a nonpolar solvent able to extract nonpolar lipids [31, 33, 42, 44, 45, 47, 48]. However, it is important to remember that polarity is not a binary property but a spectrum ranging from nonpolar to extremely polar. As such, many researchers have labeled carbon dioxide as a low polarity solvent that will extract nonpolar to very low polarity solutes [14, 17, 23, 38, 39, 49]. A few works have provided a summary of six characteristics of $SCCO_2$:

> "(i) it dissolves non-polar or slightly polar compounds;
>
> (ii) the solvent power for low molecular weight compounds is high and decreases with increasing molecular weight;

(iii) $SCCO_2$ has high affinity with oxygenated organic compounds of medium molecular weight;

(iv) water has a low solubility at temperatures below 100°C;

(v) proteins, polysaccharides, sugars and mineral salts are insoluble; and

(vi) $SCCO_2$ is capable of separating compounds that are less volatile, have a higher molecular weight and/or are more polar, as pressure increases" [9, 50].

The compounds that pure $SCCO_2$ is known to extract are: glycerides [9], free fatty acids [51], carotenoids and chlorophyll *a* [20, 38, 39], cholesterol [23], phenolics [33], terpenes [52], and xanthophylls [20]. It is interesting to note that polar phospholipids are not extracted with $SCCO_2$ [53]. To accomplish the extraction of more polar compounds a polar entrainer would need to be added to the $SCCO_2$.

It is important to not only to specify the types of compounds that are extracted but to also include the compounds necessary for biofuel production. The glycerides, free fatty acids, phospholipids, and cholesterols all contain fatty acid chains which are of interest as stated before. The usefulness of the lipid profile for biofuels is dependent on the saturation. The more unsaturated the lipid is, the more susceptible it is to oxidation. Lipids with four or more degrees of unsaturation are likely to not only be oxidatively unstable but to also have poor volatility, a high cloud point, and gum formation [54-56]. The lipid profile is known to be affected by the culture conditions, especially for algae, but how the extraction conditions of $SCCO_2$ affect the lipid profile is not as well known [3, 4, 24, 25, 27, 28]. Of the research reviewed for this thesis, only a few presented a lipid profile as part of the extraction data for $SCCO_2$. Some of those works were in

agreement that the extraction conditions do not affect the lipid profile of the extract [16, 31, 44]. Whereas others concluded that the lipid profile is affected by the extraction conditions [34, 35, 55]. However, reported lipid profiles did not have a wide range of variation. It appears that the majority of the lipid profile differences came from the extraction pressure. At low pressures there is a wide diversity of low concentration FAMEs and at high pressures their concentration is even lower or nonexistent [55]. It is important to note that the $SCCO_2$ extraction pulls out whole glycerides and other transesterifiable materials. Then the sample is transesterified to FAMEs in order for the lipid profile to be measured and reported. The solubility of the glycerides and other molecules may not be influenced as much by the extraction conditions since the fatty acid chains are only part of the whole molecule. It is possible to conclude that the bulk of the lipid profiles are consistent for $SCCO_2$ extraction conditions, but the molecules containing the low concentration fatty acids are more sensitive to the extraction conditions.

Overall, using $SCCO_2$ extraction on microalgae could prove to be a valuable source of FAMEs for biofuels. The lipid composition can be favorably altered in microalgae based on stressors [3, 4, 24, 25, 27, 28] with the majority of the lipids being nonpolar triglycerides [4, 5, 27]. These nonpolar triglycerides can be extracted by $SCCO_2$ and then transesterified into biodiesel [7]. Currently there does not exist a refined and rigorous understanding of the extraction kinetics of $SCCO_2$ extraction on algae but this thesis aims to fill in these knowledge gaps.

II. Preliminary Work: Pilot Study

Introduction

The first set of experiments was part of a "Pilot Study" into supercritical CO_2 extraction. These first experiments were used as a first exploratory endeavor. The main purpose was to become familiar with the apparatus and to demonstrate that algal lipids can be removed with supercritical CO_2. The experimental plan was to test the edges of the variable space, and operational limits of the apparatus. This exploratory testing would provide a large range of potential results at the cost of low resolution. The planned analytics for this study was a gravimetric measurement at the end of each run, along with a thin layer chromatography (TLC) and gas chromatography (GC) of the final extract.

Apparatus

The apparatus used for this study was designed and manufactured by Eden Labs (Eden Labs LLC., USA). A sketch of the apparatus is shown in Figure 2 (a photo of the actual apparatus is in Appendix). The unit consists of two pumps, a back pressure regulator, a heat exchanger, various ball valves, and four pressure vessels. These pressure vessels are the storage tank, extractor, and two separators. The storage tank acts as a capacitance against the cyclic action of the pump. The extractor is where the algal sample is loaded for extraction. The two separators are where the extracted material is precipitated out of the CO_2 depressurization. Each of the pressure vessels has a fiberglass

insulation wrapped Watlow HC05-129 heater (Watlow Electric Manufacturing Co., USA) to heat the outside of the vessel which helps maintain the temperature inside the vessel. The pressure vessels also each have an Omega type J thermocouple (Omega Engineering Inc., USA) and a LaMOT rupture disk (LaMOT, USA). The top of the storage tank, extractor, and second separator are equipped with a pressure gauge. The heat exchanger between the storage tank and the extractor is connected to a VWR Signature Chiller (VWR International LLC., USA). The carbon dioxide is supplied by a CO_2 cylinder from Airgas (Airgas Inc., USA). The apparatus also has a Hydraulics International 5G-SD-100 pump (Hydraulics International Inc., USA) that increases the CO_2 pressure from the pressure inside the cylinder to the desired extraction pressure. There is a second pump, Hydraulics International 5LG-TS-4, which is not used during extractions, but rather to evacuate the system of ambient air before an extraction after the algal sample is loaded. The pumps are air driven with the utility air supplied by an Ingersoll-Rand compressor (Ingersoll-Rand plc, Ireland). The utility air passes through a Norgren Excelon F73 filter and R73 regulator (Norgren Ltd., UK) before it reaches the pumps. The back pressure regulator drops the extract laden CO_2 from the extraction pressure to the CO_2 cylinder pressure and is a Swagelok KHB series regulator (Swagelok Co., USA). The flow path of the CO_2 is stainless steel tubing from Swagelok and the numerous ball valves are Hy-Lok H1B series (Hy-Lok Corporation, China). The flow path can be redirected by the ball valves to allow top-to-bottom or bottom-to-top flow in the extractor, but simply put the CO_2 flows from the storage tank to the extractor in any configuration.

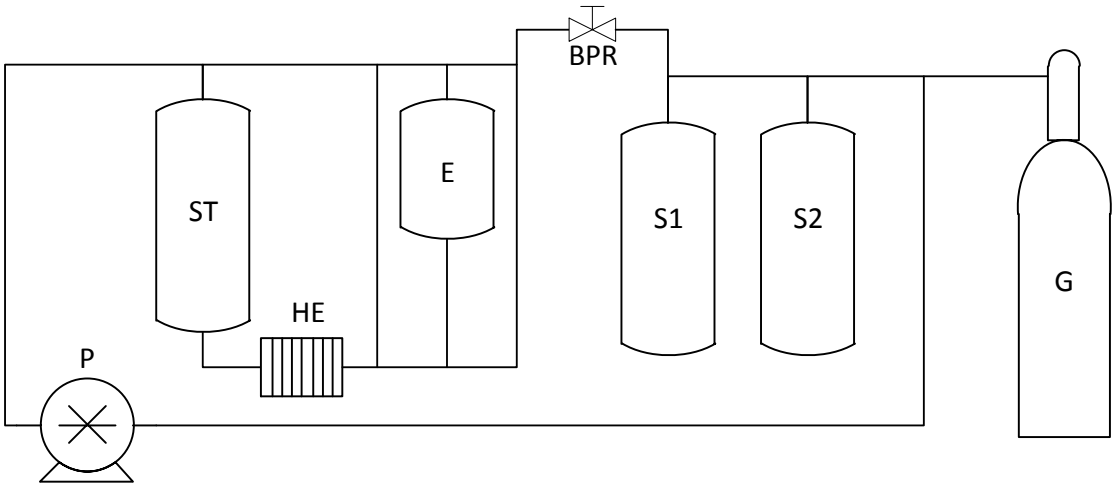

Figure 2. Sketch of the apparatus flow-path for the Pilot Study. P= pump, ST= storage tank, HE= heat exchanger, E= extractor, BPR= back pressure regulator, S1 and S2= separators, G= CO_2 cylinder. The ball valves have been omitted for clarity.

Methods

The microalgae used for the experiment was kindly provided by Solix BioSystems (Solix BioSystems Inc., USA). The algae strain is *Nannochloropsis oculata;* at harvest it was measured to have 17.5% FAMEs by dry mass. The algae were received frozen and partially dewatered at 74% water by mass. The algae were placed in an oven at 100°C until fully dry. Once all the water was removed, the algae went through a few steps of size reduction. First, the algae was broken into manageable pieces by hand to be further reduced in a mortar and pestle, which allowed the algae to then be ground in a Black & Decker CBM210 burr mill (Black & Decker, USA). Once ground, the algae was sieved (VWR International LLC, USA) to have a diameter of 300 μm to 710 μm. The dried and ground algae were then vigorously homogenized. Once homogenized, small containers were filled with approximately 85g of dried ground algae; the approximate

maximum capacity of the extractor. Each container was stored under nitrogen or argon at -4°C until use.

The preparation steps taken before an extraction were modified with each run conducted. Initially all the runs started with loading the algae sample into the extractor, sealing the extractor, evacuating the system, and then introducing the CO_2. After this point the standard procedure for preparation was tweaked as familiarity with the apparatus was developed. These tweaks involved adding a partial bypass of the extractor to initially allow the system to reach the test conditions before extraction occurred. A leak check of all connections prior to extraction as also added due to a leak in one of the experimental runs. Once these preparatory steps were taken, the extraction was conducted.

At the end of an extraction, the vessel heaters and pump were turned off and the system was clean out and the extract collected. The first step was to exhaust the CO_2 from the system. Then the algae sample was removed from the extractor. Once the separators reached a temperature that allowed for handling, the separators, tubing from the back pressure regulator to the first separator, and the tubing in between the separators were thoroughly rinsed with hexane and then methanol. These solvents where chosen because $SCCO_2$ is commonly compared to hexane as a nonpolar solvent, and the methanol was a strongly polar solvent that should catch any remaining solutes that were not soluble in hexane. The hexane and methanol rinse was collected in a glass flask which was ready for the rotor-evaporator.

A concern arose about possible extract remaining in the system that was not collected through the cleanup method. This extract could possibly be contained in the back pressure regulator, tubing before the back pressure regulator, or in tubing after the second separator. Due to this concern the practice of "blank runs" was incorporated. A blank run involved operating the system normally, but without an algae sample. The extracts collected from these blank runs were only analyzed gravimetrically.

The first method of analysis was a gravimetric analysis of the extract. This was accomplished by weighing the empty rotor-evaporator ready flask before the extraction. Once the extraction was completed and the methanol and hexane rinses were added to the flask, it was place on a Büchi rotor-evaporator (Büchi Labortechnik AG, Switzerland). The conditions for solvent removal were set at 50 mbar of pressure and the temperature was 60°C (unless the extraction temperature was lower, in which case the rotor-evaporator temperature matched that of the extraction temperature). Once all the solvent was removed, the flask was weighed again, and the mass difference was determined as the extract mass.

The second method of analysis was a thin layer chromatography (TLC). This involved diluting a small portion of the extract, about one drops worth, in 1 mL of a chloroform : methanol (2:1) solution. The dilution was spotted onto a Whatman silica-gel plate (Whatman International Ltd., England). The first two treatments were only for the bottom half of the plate using a solution of chloroform : methanol : water (60:30:5). The third and final treatment was of the whole plate using a solution of hexane : diethyl ether : acetic acid (80:20:1.5). After each treatment the plate was allowed to air dry in a fume hood for approximately 20 minutes. Once completely dry the plate was sprayed

with a solution of copper sulfate : orthophosphoric acid : water (1:1:10). This spray was to aid in the development of the spots during charring [57]. The plate was then charred with a heat gun (Milwaukee Electric Tool Corporation, USA) until the lipid spots turned a dark color, but before any charring of the silica gel. TLC is capable of a qualitative and quantitative analysis provided that the exact amounts are known in the initial spot and a thorough a densitometry technique. However, for this piece of work a quantitative method did not seem practical based on the resources available. The TLC analysis in this work was purely used for a qualitative analysis.

The final method of analysis performed was gas chromatography (GC) using the Solix developed method [58]. This method involved weighing out a known mass of the extract into a vial. The sample was transesterified in methanol with a potassium hydroxide catalyst. The transesterification was allowed to progress for 30 minutes with periodic homogenizing. The reaction was quenched with a stoichiometric amount of acetic acid. The mixture was then extracted against heptane and centrifuged to separate the heptane and lipid layer from the methanol, glycerol, water, and salt. The lipid-heptane was then diluted in heptane with an internal standard (for calibration) to a specified dilution amount. The samples were then loaded onto the Agilent 7890 GC (Agilent Technologies, USA). The machine has a split-splitless injector, operated in splitless mode, with a flame ionization detector. The column is a Restek FAMEWAX (Restek Corporation, USA) 30 m long with a 0.32mm internal diameter. The carrier gas was helium. For each sample run, the oven was initially at 90°C for 30 seconds then heated at: 70°C/min until 208°C, 3°C/min until 230°C, and 2°C/min until 240°C and held at that temperature for 30 seconds. A computer interpreted the data into

chromatograms and calculated amounts for each peak detected based on the calibration curve for the run.

Results and Discussion

There were a total of six extractions and three blank runs conducted in this pilot study. The test conditions and mass of extract are shown in Table 2. The table does not show the target conditions but the average value for the parameter along with the standard deviation. The runs are listed in chronological order and the naming scheme refers to whether the run was an experimental "Pilot Run" or a "Blank Run". After the last extraction cleanup (PR-6) the system was completely disassembled and all the tubing, vessels, and components were cleaned out with hexane and methanol. This teardown revealed that there was a significant amount of material remaining in the system. The material was found lining some of the tubing, spaces in the ball valves, in the back pressure regulator, and the inlet and outlet of the pump. This would suggest that some of the extract carried over past the separators, but based on the very green color of the majority of the material, its very likely that most of it was actually fine algae particles. The extractor was fitted with a stainless steel frit at both the top and bottom, but those only held back particles larger than 50 μm in diameter. Although the algae was sieved to be between 300 μm and 710 μm there is still a possibility that some of the fine particles stayed stuck to the larger particles, then during the extraction were knocked lose and carried throughout the system.

Table 2. Conditions for extractions and blank runs for the Pilot Study. *These masses were weighed on a balance with 0.5g precision.

Run Name	Pressure (bar)	Temperature (°C)	Duration (h)	Algae Sample (g)	Extract (g)
PR-1	110 ± 7.0	37 ± 7.9	1.700	85.5*	3.0*
PR-2	230 ± 74	32 ± 11	1.367	85.0*	4.712
PR-3	350 ± 7.4	50 ± 12	3.633	86.112	9.001
BR-1	390 ± 14	20 ± 1.7	1.000	0.000	0.225
PR-4	350 ± 3.4	60 ± 4.1	4.333	83.946	8.613
BR-2	360 ± 4.5	85 ± 5.1	1.000	0.000	0.974
PR-5	350 ± 2.5	66 ± 5.2	3.183	85.148	6.839
BR-3	400 ± 31	74 ± 15	2.000	0.000	0.578
PR-6	100 ± 1.2	90 ± 3.7	4.000	82.916	0.513

As described before there were three main methods of analysis. Of the three methods of analysis, the hardest to relate to the literature proved to be the gravimetric weighing of the samples collected. Although final masses were found for each Pilot Run, comparing them directly to the literature would be misrepresentative. According to the literature the extraction is not linear process but is a root curve [16, 20, 31-33, 35-37, 40, 43, 45, 48, 55, 59]. Meaning that only knowing the starting amount of extract and the final amount is not enough information to make an accurate curve for the extraction conditions. However, there does seem to be a correlation between the severity of the conditions and the amount of extract collected, albeit the duration of the extraction was different for each run. The collected masses seem to be in agreement with the notion from literature that at a constant temperature, a pressure increase yields an extract increase as shown from PR-1 to PR-2. Similarly the second notion from literature stating that for a constant pressure, a temperature increase would yield a decrease in extract was seen from PR-4 to PR-5 to PR-6. The variation in duration makes it difficult to verify with certainty that this trend was observed.

The next method of analysis of the samples was TLC. This analysis was used to show the presence of any polar compounds, since the literature suggests that $SCCO_2$ extracts only nonpolar and very slightly polar compounds at less severe conditions, then slightly more polar compounds at more sever conditions. A photo of the TLC plate for the Pilot Study is shown in Figure 3. To interpret the TLC plate it is important to remember that polarity is not a binary property; it is a spectrum. The middle line marks the separation of the top "nonpolar" half and the bottom "polar" half. The closer the spot is to the starting line the more polar the compound is. The first lane is a chloroform : methanol extract that was provided by Solix BioSystems to be used as a reference to demonstrate what a sample containing nonpolar and polar compounds would look like. Lanes 6 and 9 are material that was removed from the apparatus as part of the complete teardown. The remaining lanes are the experimental runs of the Pilot Study. Figure 3 reveals the nonpolar nature of $SCCO_2$ as a solvent. This is in agreement with the literature as described before that $SCCO_2$ is a nonpolar solvent that will extract nonpolar and slightly polar compounds [9, 14, 17, 23, 38, 39, 49, 50]. It is also noteworthy to point out that the nonpolar spots in the chloroform : methanol extract correlate well to the $SCCO_2$ extract. If a standard of known compounds was used on the plate then the spots of the $SCCO_2$ extraction and chloroform : methanol extraction could be compared to the standards spots. This would have provided more detailed information about the compounds extracted. Unfortunately, a standard was not used on this plate.

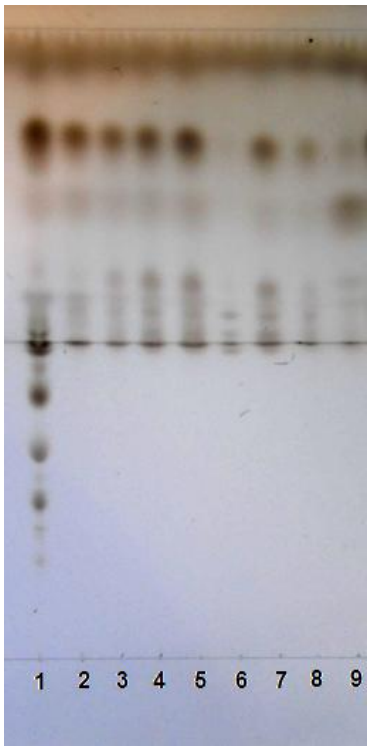

Figure 3. TLC plate for Pilot Study. 1 = chloroform : methanol (2:1) extract of algal sample, 2 = PR-1, 3 = PR-2, 4 = PR-3, 5 = PR-4, 6 = sample from final cleaning, 7 = PR-5, 8 = PR-6, 9 = sample from final cleaning. (Photo courtesy of Steve Bunch, Solix Biofuels).

The final method of analysis was GC, with each pilot run sample analyzed in triplicate. It is important to remember that the GC measures FAMEs that could have come from a number of compounds such as glycerides, cholesterol, and phospholipids. Since the TLC analysis showed the $SCCO_2$ extraction did not extract polars, it was assumed that the polar phospholipids were not present in the extract. This leaves the glycerides and cholesterols as the only source of the transesterifiable esters. Figure 4 shows the FAME profile for each of the pilot runs. As can be seen, the dominant chains are 16:0, 16:1, and 18:1, with the first number representing the number of carbon atoms in the chain and the second number representing the number of double bonds. The extraction conditions did not seem to affect what chains were present, but only the

relative amount of each chain. This was expected based on the assumption that any ester position on the molecule could be any fatty acid chain that the algae contained. The variation in the relative amount of each FAME could be attributed to the overall molecule's solubility at different extraction conditions, which is partially dependent on the fatty acid chains attached. What can be concluded from Figure 4 is that with a pressure and temperature increases there is a corresponding decrease in the 16:0 chain and an increase in the 18:1 chain. The chain 22:2 also appears to have the largest relative amount when extracted at a low pressure and high temperature. It is also interesting to note that at a constant high pressure, a temperature increases only results in a slight increase of 20:5 and a slight decrease of 18:1. Another way to interpret the GC data is in the form of saturation versus unsaturation. Table 3 shows the relative degrees of unsaturation for each pilot run. From a biofuels stand point it is important to account for the amount of unsaturation as there is a tradeoff between cloud point and oxidative stability. If a low cloud point is desired then low-pressure and high-temperature yield the most relative amount of unsaturation. If the desire is for oxidative stability then low-pressure and low-temperature yield the most relative amount of saturation.

Table 3. Relative amounts of saturation and unsaturation for pilot runs. Conditions for each run are shown as mean values.

	PR-1 (110 bar @ 37°C)	PR-2 (230 bar @ 32°C)	PR-3 (350 bar @ 50°C)	PR-4 (350 bar @ 60°C)	PR-5 (350 bar @ 66°C)	PR-6 (100 bar @ 90°C)
% Saturated	36.53	33.72	31.55	30.95	31.13	30.28
% Unsaturated	63.47	66.28	68.45	69.05	68.87	69.72
% Mono-unsaturated	42.74	41.74	41.09	41.80	41.50	38.98
% Poly-unsaturated	20.72	24.54	27.35	27.24	27.37	30.74
% Saturated / % Unsaturated	0.58	0.51	0.46	0.45	0.45	0.43

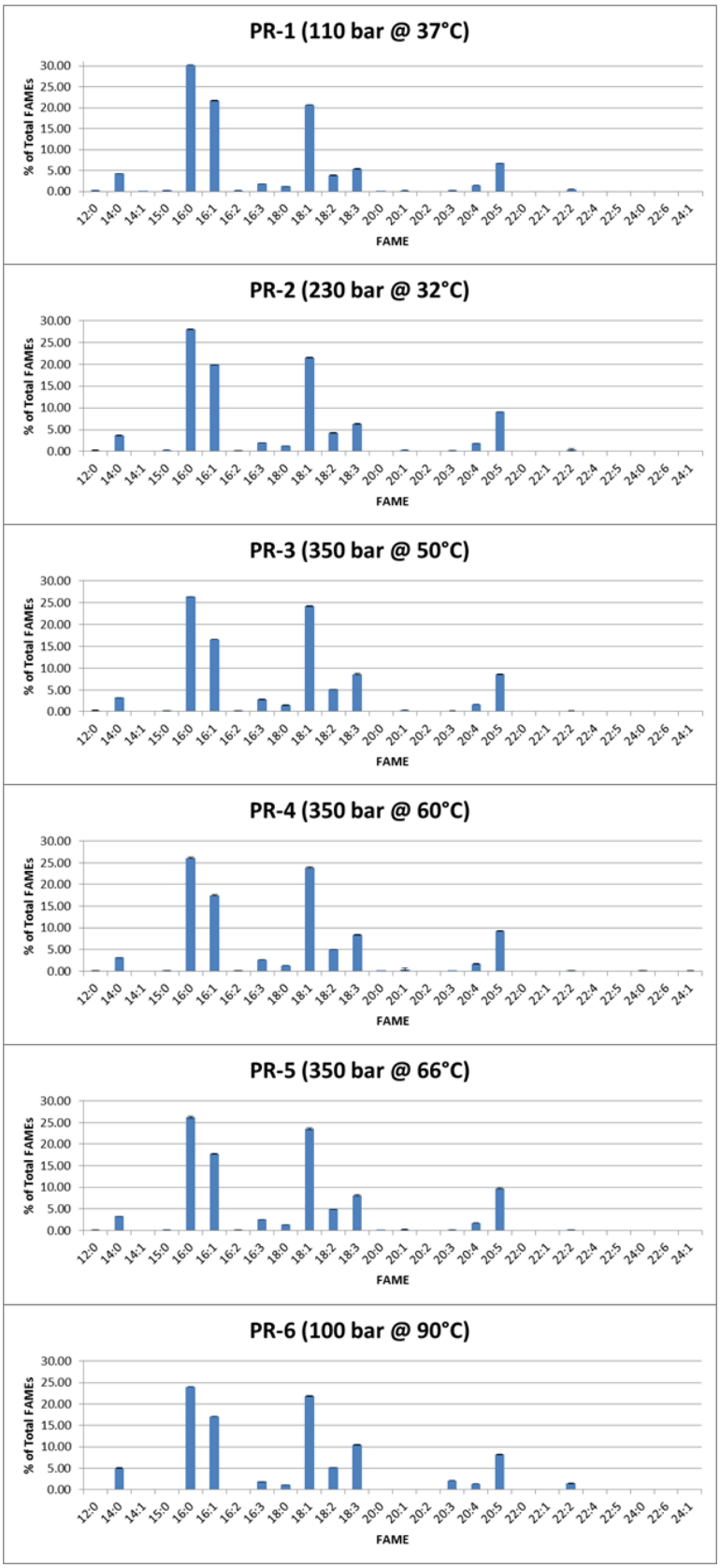

Figure 4. Results from GC analysis showing the FAMEs profile of the pilot runs.

Some of the knowledge gained from the Pilot Study was not from the data collected, but from the operation of the apparatus. As stated before there was a problem of fine particles of residual algae and extract in the system after an extraction. The blank runs were an attempt to remove these residuals, but it was clear after the final cleaning that these blank runs did not effectively remove all of them. These residuals could not only alter analytical results by being a contaminant, but could also allow for the under- or over-measurement of the extract depending whether or not they ended up in the separators. These residuals could also damage the system's components. The pump is not rated for particulates, nor is the back pressure regulator. Another concern was the lack of measurement of the CO_2 being utilized for the extractions. The total amount of CO_2 and the rate at which it was flowing through the extractor was not known. These parameters would be of interest to better understand a $SCCO_2$ extraction. These concerns raised the need to modify the apparatus for future experiments.

Conclusion

The Pilot Study was the first experiment conducted in $SCCO_2$ extraction. Its exploratory nature yielded experimental results and operational deficiencies. The experiments showed that the conditions yielding the most extract were 345 bar and 50°C, although the problem of residual algae particles and extract could have altered which run produced the most extract. The TLC analysis showed that for the conditions tested, $SCCO_2$ only extracted nonpolar material. The FA profile of the extract, as measured as FAMEs on the GC, showed that the dominate chains were 16:0, 16:1, and 18:1. There was a trend of a decrease in 16:0 and an increase in 18:1 with an increase in pressure and temperature. Interestingly, only the low pressure and high temperature extraction

conditions produced the largest amount of 22:2. The conclusion achieved from this analysis is that the FA profile changes slightly with the extraction conditions. This is most likely due to the small change in solubility of the whole molecules with differing test conditions. The Pilot Study revealed that future experiments in $SCCO_2$ extraction needed to address the issues of residual algae fines and extract in the system. As well as including a way to measure the total amount of CO_2 or the flow rate of the CO_2 through the extractor.

II. Preliminary Work: Equilibrium Study

Introduction

This Equilibrium Study was intended to build on the operational improvements presented by the Pilot Study, including a way to measure the CO_2 utilized during an extraction and to prevent carry over into the rest of the system. This required a reconfiguration of how the apparatus was operated. Fortunately, the apparatus is capable of such modifications. The goal of this study was to measure the CO_2 used during the extraction. This was accomplished by allowing the extractor loaded with algae and CO_2 to remain sealed off from the rest of the system until the oil in the algae came into equilibrium with the oil in the CO_2. This provided a way to measure the maximum amount of oil attainable at an extraction condition because it allowed for the avoidance of mass transfer resistance associated with a CO_2 flow. The intention was that the measurement of the CO_2 and an equilibrium extraction would allow the capabilities of $SCCO_2$ extraction to be better explored.

Apparatus

The apparatus used for the Equilibrium Study was the same as for the Pilot Study with the exception of several modifications. These modifications included reconfiguring the tubing between the storage tank and the extractor, along with adding a new flow path out of the extractor. Figure 5 shows that the heat exchanger was kept but the flow through the extractor could only be top-to-bottom. A Swagelok HN series needle valve

(Swagelok Co., USA) was added to the outlet of the extractor. The needle valve was in place to step down the CO_2 from extraction pressure to ambient pressure. After the needle valve, two 2L glass flasks were used in series as collectors for the extract. As a safety measure the glass flasks were housed in a polycarbonate box in case of over pressurization. The polycarbonate box was also able to hold water which could be utilized to regulate the temperature of the collectors. A plastic bag was placed at the outlet of the second collector with a ball valve on the only port of the bag. This bag was modeled after a tube of toothpaste and was used for capturing and holding all of the CO_2 released from the extractor. The final addition to the apparatus was a graduated carboy used to measure the captured CO_2. Photos of the actual apparatus can be seen in the Appendix.

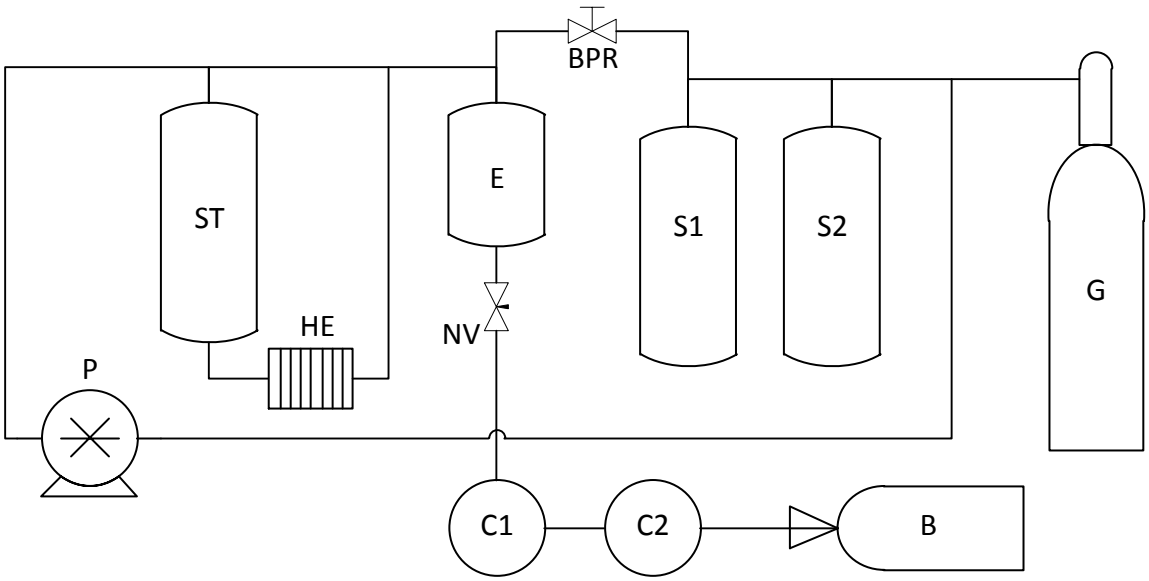

Figure 5. Sketch of the apparatus flow-path for the Equilibrium Study. P= pump, ST= storage tank, HE= heat exchanger, E= extractor, BPR= back pressure regulator, S1 and S2= separators, G= CO_2 cylinder, NV= needle valve, C1 and C2= collectors, B= CO_2 collection bag. The ball valves have been omitted for clarity.

Methods

The preparatory steps taken for the Equilibrium Study were similar to the Pilot Study. The algae was the same batch as used and described in the Pilot Study. The extractor was loaded with the algal sample, then the extractor was sealed, the system evacuated, and the CO_2 introduced. The pump was turned on and vessel heaters were set to the desired temperature. Once the extractor reached the desired conditions a ball valve upstream from the extractor was closed and the pump was turned off. This sealed off the extractor and some tubing upstream and downstream of the extractor, from the rest of the system. The algae sample then sat in the extractor with the CO_2.

Once the extractor had been sealed at test conditions for an hour, several steps were taken to collect the extract sample. First, a small amount of the CO_2 was relieved through the needle valve. The amount relieved was intended to mostly inflate the CO_2 collection bag. Once the collection bag was deemed full, the ball valve on the bag was closed along with the needle valve. Next the pump was turned on and the ball valve upstream of the extractor was opened until the desired pressure was reached again in the extractor. Once the desired pressure was attained, the ball valve was closed and the pump turned off. The extractor was then sealed and allowed to sit for another hour. To collect the extract sample the collectors were removed from the polycarbonate box. The collectors, tubing from the needle valve to the collectors, tubing between collectors, and tubing from the second collector to the collection bag were all rinsed with hexane and methanol. The rinse was collected in a rotor-evaporator ready flask for each sampling. The collector assembly was then reassembled and ready for the next sampling.

At the end of an extraction run the CO_2 was exhausted and the vessel heaters turned off. The collector assembly was cleaned out as described. In addition the needle valve was rinsed with hexane and methanol. The algae was also removed. The flasks with the extract and rinse would then follow the same methods of analysis as did the samples for the Pilot Study. The one alteration to the methods is that the TLC analysis had a standard added in order to compare the extract spots to the standard's spots of triglyceride, free fatty acid, diglyceride, and monoglyceride.

Results and Discussion

There was only a single equilibrium run conducted for this study. The reason is that the setup had a potential for precise data on the maximum amount of lipids extracted at a specific condition, but at the cost of an extremely long duration. The first difficulty was having enough rotor-evaporator ready flasks. There were a limited amount, requiring them to be reused throughout the study. This caused an increase in the time it took to complete the equilibrium run as the few flasks would be used and the samples they contained would need to be analyzed before the flasks could be cleaned and reused. The analysis and cleaning would add a day's worth of work in between collecting samples from the equilibrium run. This added time to the already lengthy extraction duration made this setup impractical.

The Equilibrium Study arrangement did eliminate the operational issues that were presented with the Pilot Study configuration. After the Equilibrium Study there was a complete tear down and clean out of the entire system, as with the Pilot Study. This clean out revealed there were no algae fines or extract that travelled from the extractor to

the back pressure regulator, separators, or to the pump. However, there was a new problem introduced with the Equilibrium Study configuration. The pump would only be turned on for a very short duration and then would remain off for the majority of the experiment. These very short duty cycles are not optimal for the pump as it was designed with the intention of continually running. These short cycles would sometimes cause the pump to stall. Although the stalls were easily remedied, it still provided an inconvenience. Future experiments needed to take this into account.

Similar to the Pilot Study the Equilibrium Study underwent the same three methods of analysis. The gravimetric analysis for the single equilibrium run is shown in Figure 6 as a plot of the extraction ratio versus the amount of CO_2 used during the run. The extraction ratio is the ratio of the extract mass over the mass of the initial biomass. It was chosen to present the extract mass in this way as it normalizes any slight variation in the amount of initial biomass between runs. (Although there was only one equilibrium run conducted, it is useful to present the data in a form that allows for easier comparison to other experiments.) The error bars for the extract ratio came from the amount of extract remaining in the system, from extractor through needle valve, after the equilibrium run. The three gaps in the curve came from an attempt to expedite the extraction by allowing an open flow of CO_2 through the extractor without collecting the CO_2. To account for this unmeasured CO_2, a linear interpolation based on the ratio of CO_2 mass to the mass of the extract fraction was conducted in order to fill in the missing data points in Figure 6. The shape of the curve shows how the beginning of the extraction appears to follow a linear curve, then becomes more of a root curve, and finally appears to level off. The gravimetric analysis for the equilibrium run has shed

some more light onto $SCCO_2$ extraction. The extraction of mass is not a linear relationship but a curve, which is in agreement with the literature [16, 20, 31-33, 35-37, 40, 43, 45, 48, 55, 59].

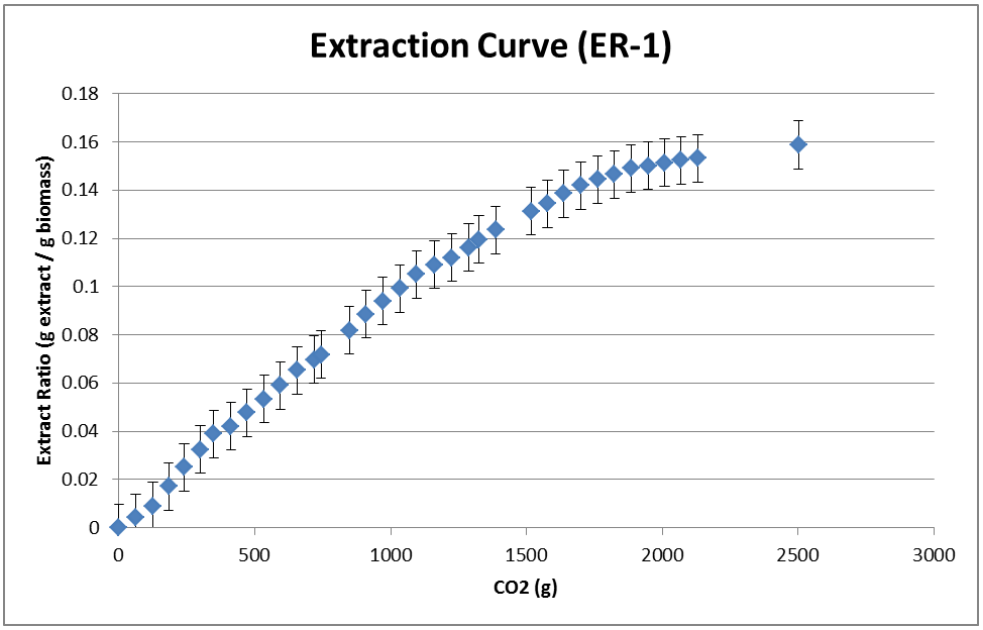

Figure 6. Extraction curve for Equilibrium Run ER-1, 410 ± 31 bar at 100 ± 0.1 °C.

The second method of analysis was TLC. As stated previously, the method included a standard with the extracts on the TLC plate. Figure 7 shows the results from the TLC analysis. The center lane "S" is the standard and the numbers represent the fraction number in chronological order. The method used allowed for a quick identification of glycerides, cholesterol, cholesterol esters, and free fatty acids [60, 61]. The standard was only used for glycerides and free fatty acids. The results show that all of the extract was above the halfway line meaning they are nonpolar. This concurs with the literature [9, 14, 17, 23, 38, 39, 49, 50]. It is interesting to note that there are more spots present in the extract than in the standard. The very top spot, which is nearly at the

solvent line, is known to be cholesterol esters, and the faint spot between the free fatty acid and diglyceride is known to be cholesterol [60]. The apparent split of the diglycerides and monoglycerides in the extract and standard were not determined, but it is known that the split comes from the location of the fatty acid chain(s) on the glycerol backbone. In chromatography it is common that the 1,2 diglyceride and 1,3 diglyceride do not elute at the same rate [62-65]. This could be extended to the monoglyceride as well; that the location of the fatty acid chain on the glycerol affects the elution rates [66].

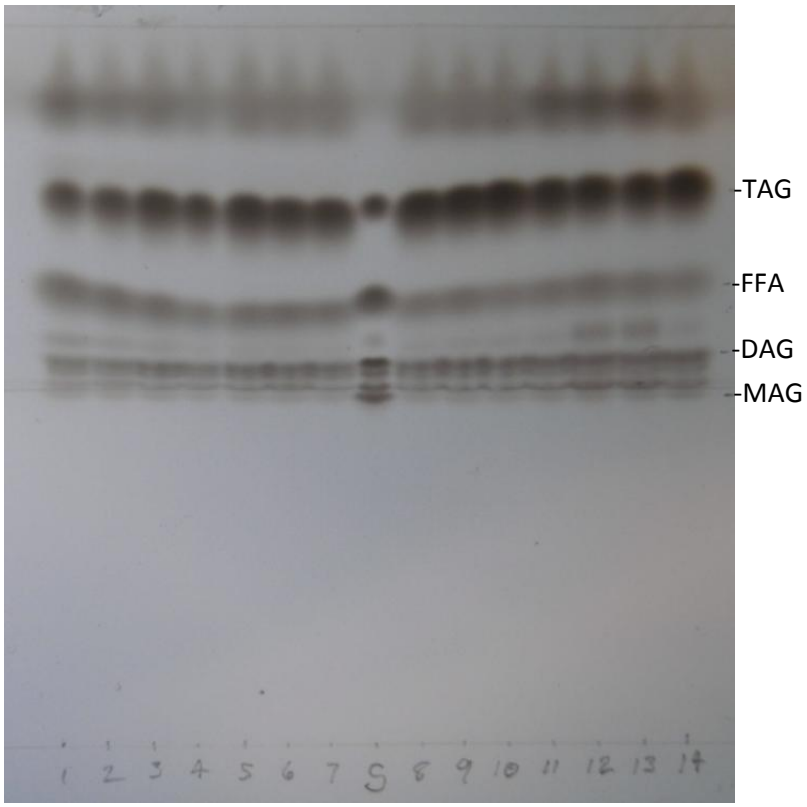

Figure 7. TLC plate for Equilibrium Study run ER-1 for the first 14 fractions. Key on the side denotes the spots for the standard: TAG = triglyceride, FFA = free fatty acid, DAG = diglyceride, and MAG = monoglyceride.

The third and final analysis of the Equilibrium Study was GC. The design of the Equilibrium Study resulted in the ability to measure the lipid profile over the course of the extraction. This information had not been found in the literature at the time of this

experiment. The lipid profiles, as shown in Figure 8, revealed that the relative amounts of many of the chains end similar the start of the extraction. There was no clear trend for the saturated chains; as 14:0 and 16:0 decreased and 18:0 and 20:0 remained around the same value. Similarly there was no trend when examining the unsaturated chains as a whole. However, when the degree of unsaturation was examined, a trend emerged. The mono-unsaturated chains decreased throughout the course of the extraction, with the exception of 18:1 increasing. The double and triple unsaturated chains appeared to follow the trend of remaining approximately constant with a possible decrease in the relative value. The very polyunsaturated (four and five degrees of unsaturation) chains appeared to have a separate trend of increasing throughout the course of the extraction. This is evidence that the extraction duration may affect the lipid profile over the course of the extraction. The trends can be summarized as saturated, one, two, and three degrees of unsaturation lead to the lipid's relative amount remaining approximately constant or decreasing throughout the extraction. Lipids with four or five degrees of unsaturation have their relative amounts increase throughout the extraction. This could be due to the fact that the higher degrees of unsaturation lead the chain to becoming slightly more polar in nature from the uneven charge distribution of the double bonds. This would then affect the nature of the entire molecule that the highly unsaturated chain is attached to. The end result being that the molecules containing highly unsaturated chains are not as soluble as the lesser unsaturated or saturated lipid containing molecules. The saturated and lesser unsaturated molecules would then be extracted slightly faster than the highly unsaturated. This would lead to the increase in the relative amount of highly unsaturated molecules toward the end of the extraction.

However, it is important to remember that the increase in highly unsaturated lipids is a relative measurement. The absolute amount, as shown in Figure 6, diminishes quickly towards the end of the extraction. The 30th fraction had a relative amount of 13.71% of 20:5 FAMEs, which is double the relative amount in the 1st fraction of 6.89%. This means that to double the relative amount of 20:5 in the extract fraction, about 92% of the extract was already removed. From a biofuels stand point, limiting the extraction duration to a shorter time would reduce the relative amount of the oxidatively unstable highly unsaturated lipids [54].

More information was obtained from the GC analysis. Similarly to the Pilot Study, Table 4 shows the relative amounts of saturation and unsaturation for the equilibrium run ER-1. The values were determined by using the percentage of each FAME from the GC and applying that to the measured extract for each fraction. (This results in the assumption that 100% of the mass is transesterified into FAMEs.) The mass for each FAME was then totaled for the extraction and the final amount of FAME determined. This allowed for the calculation of the values in Table 4. As can be seen, the results of ER-1 result in comparable values as those obtained in the Pilot Study at 350 bar. The pilot run that was the most comparable to the ER-1 results, based on the values shown in Table 3, is PR-3. This is rather surprising given that there is an approximate 50 bar and 50°C difference between the extractions.

Table 4. Relative amounts of saturation and unsaturation of the total extract from equilibrium

	ER-1 (410 bar @ 100°C)
% Saturated	31.90
% Unsaturated	68.10
% Mono-unsaturated	41.21
% Poly-unsaturated	26.89
% Saturated / % Unsaturated	0.47

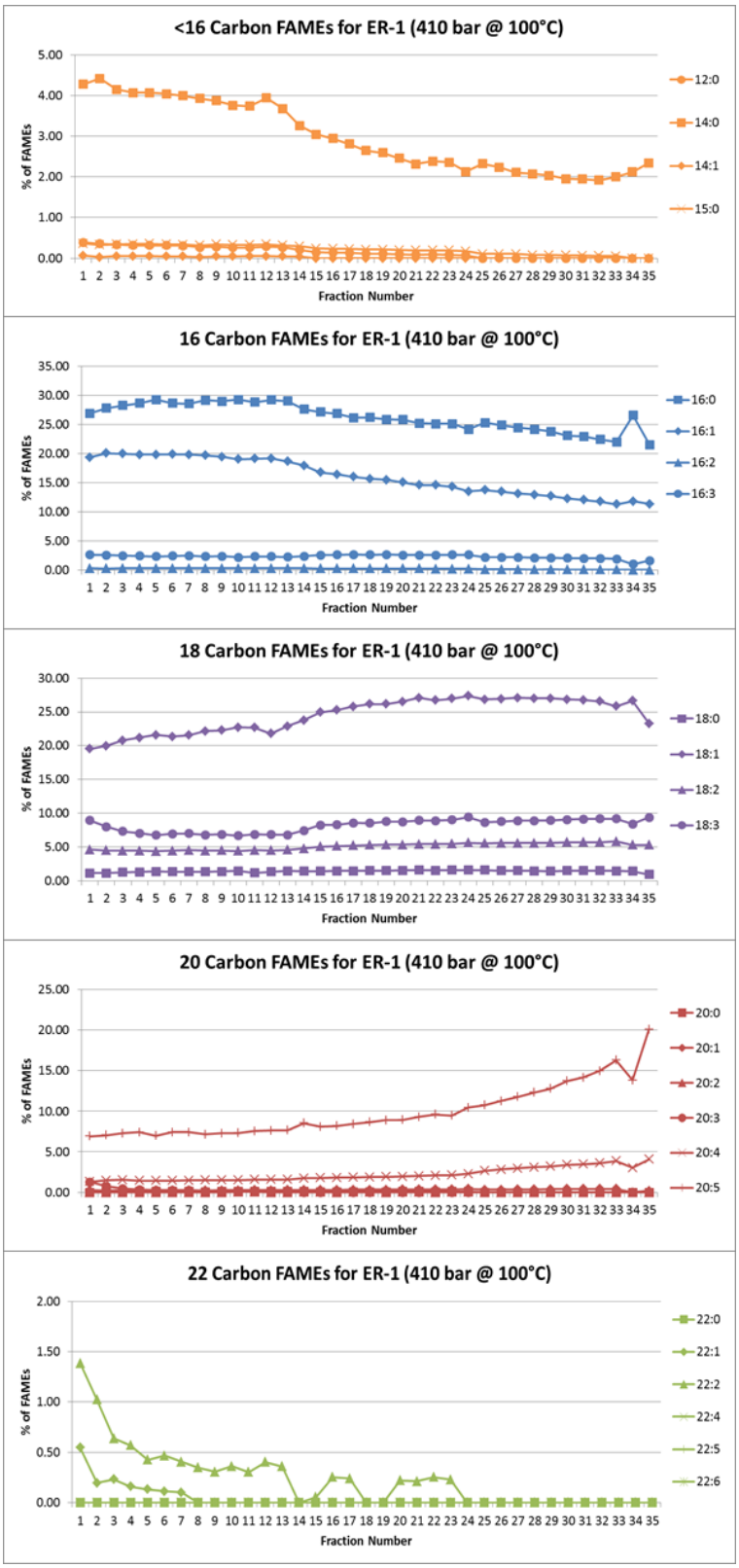

Figure 8. GC lipid profiles for Equilibrium Run 1. The following FAMEs were zero values throughout the extraction: 22:0, 22:4, 22:5, 22:6, 24:0, and 24:1.

Conclusion

The Equilibrium Study was the second experiment with $SCCO_2$ extraction. It built on the operational lessons learned from the Pilot Study. There was only a single extraction at 410 bar and 100°C which yielded a total of 13.3g of extract. The gravimetric analysis produced an extraction curve that is in agreement of the literature. The TLC analysis showed that only nonpolar material was extracted at these conditions. The FA profile of the extract, measured as FAMEs on the GC, showed that the lipid profile changed over the course of the extraction. The relative amount of saturated lipids and unsaturated lipids with one, two, and three degrees of unsaturation tended to remain relatively consistent or decrease throughout the extraction. (With the exception of 18:1 this increased throughout the extraction.) The relative amount of unsaturated lipids with four or five degrees of unsaturation increased throughout the extraction. While providing a way to precisely measure the amount of CO_2 used during the extraction, the Equilibrium Study's main drawback was the impractically long duration of extraction. For future experiments some compromise needed to be made with regards to extraction duration and accuracy of the data collected.

III. Current Work: Flow Study

Introduction

The Flow Study was intended to be the culmination of the operational lessons of both the Pilot Study and Equilibrium Study. The plan for this study was to test the corners of the pressure-temperature variable space, as was the intent with the Pilot Study. In addition there are two extraction conditions that result in the same $SCCO_2$ density, but vastly different pressures and temperatures. This was included to determine if the density is an adequate correlation to the solvating power of $SCCO_2$ as claimed in the literature [14, 35, 41, 44, 45]. The extraction conditions tested are shown in Figure 9 with the density for the CO_2 pulled from Span and Wagner [67]. The Flow Study configuration maintained a constant flow of CO_2 passing through the extractor for the duration of the extraction. This required the CO_2 measurement method, and as a result the collectors, to be changed to accommodate the constant flow. The CO_2 was measured as flow rate instead of a volume as with the Equilibrium Study. Also the tubing upstream of the extractor was reconfigured to include a high pressure bypass of the extractor. The Flow Study was a compromise between the practicality of operation and the conciseness of data collected.

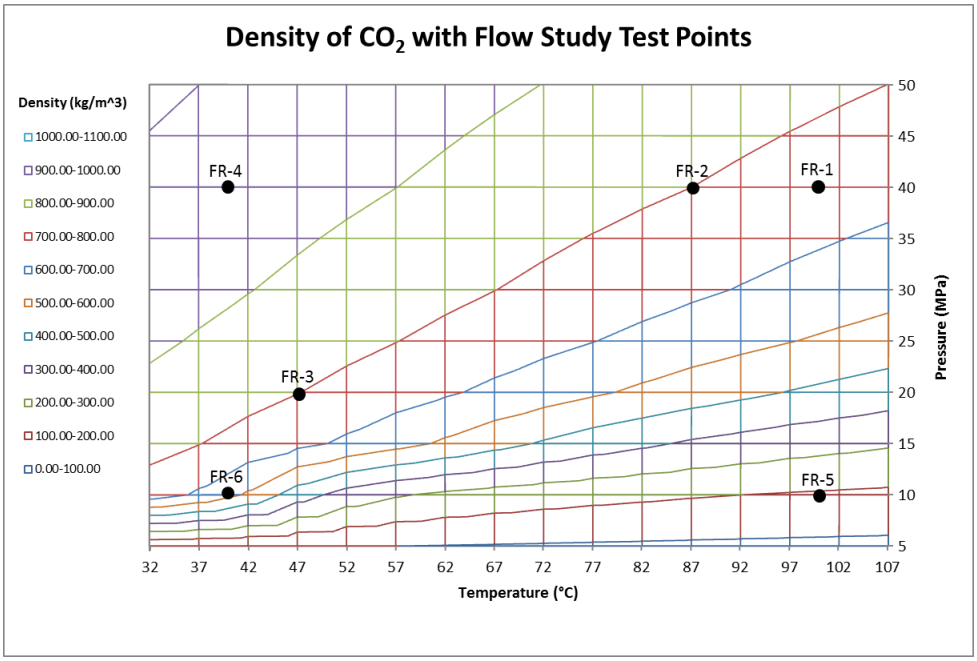

Figure 9. Overlay of the Flow Study target test points onto a contour plot of the density of CO_2. The lines represent isodensity lines.

Apparatus

The apparatus used for the Flow Study was the same as used in the Equilibrium Study and Pilot Study, with the exception of modifications to the flow path. These modifications included reconfiguring the flow path between the storage tank and the back pressure regulator. This redesign allows for a high pressure bypass of the extractor. This bypass provided two advantages; the first one being that the extractor can be completely isolated from the rest of the system by a ball valve between the bypass on extractor, and second the pump can run continuously whether the extractor assembly is open to the bypass or not. Another modification was making the outlet of the extractor at the top, with the flow path continuing to the needle valve as with the Equilibrium Study. After the needle valve, the two collectors were configured to be in parallel. There is a ball valve at the inlet of each collector to allow for independent utilization. This is one of the

main advantages of the Flow Study configuration as a collector can be in operation while the other is being rinsed clean. This allows for real-time sampling of the extract without an interruption to the extraction. After the collectors a pressure gage, thermocouple, and rotameter were installed. These three instruments allowed for the measurement of the CO_2 flow rate and provided data to convert the volume to a mass. Figure 10 shows a sketch of the Flow Study configuration, and photos of the actual apparatus can be seen in the Appendix.

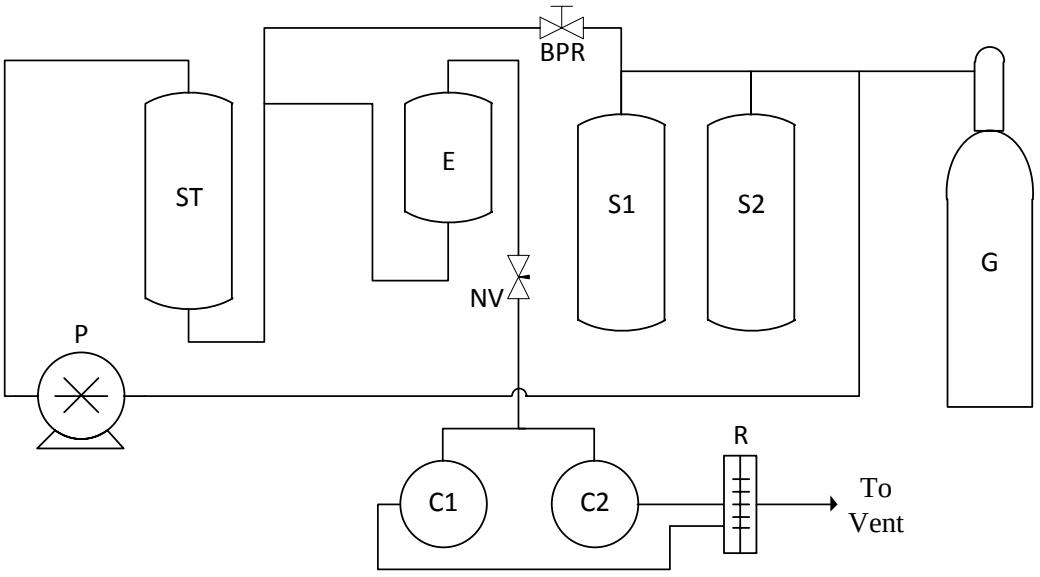

Figure 10. Sketch of the apparatus flow-path for the Flow Study. P= pump, ST= storage tank, E= extractor, BPR= back pressure regulator, S1 and S2= separators, G= CO_2 cylinder, NV= needle valve, C1 and C2= collectors, R= rotameter. The ball valves have been omitted for clarity.

Methods

The preparatory steps taken for the Flow Study were similar to the Equilibrium and Pilot Studies. The steps included loading the extractor, sealing the extractor, and introducing the CO_2. The system was not evacuated due to the fact that the flow of CO_2 would purge the system at the beginning of the extraction. For consistency, the same

algae batch was used in the Flow Study as was used in the Equilibrium and Pilot Studies. After the CO_2 was introduced, the pump was turned on and the vessel heaters were set to the desired temperature. Once the desired extraction conditions were reached the apparatus was ready for extraction.

To initiate extraction the needle valve was slightly opened and one of the collector's ball valve was also opened. (For simplicity in explanation assume C1 is the first collector utilized during operation.) The flow rate was monitored by the rotameter and adjusted by the needle valve. The CO_2 flow rate, pressures, and temperatures were recorded every 10 minutes. Every 40 minutes the valve at the non-operating collector, C2, was opened and the operating collector's, C1, valve closed. The extraction and flow was then continued in the second collector. Also, the connection of the inlet of the rotameter was attached to the outlet of the corresponding operating collector. The first collector and associated tubing with the extract would be removed and rinsed out with hexane and methanol as it was in the Equilibrium Study. Similarly the rinse was collected in a rotor-evaporator ready flask. Once rinsed, the collector would be reassembled and placed back in the system, ready for the next switch.

At the end of the extraction, the collector with the final fraction was closed, and a switch made to the other collection vessel. The CO_2 cylinder was closed and vessel heaters turned off. The flow of CO_2 was allowed to continue flowing until the system was completely exhausted. Any extract that was found in the collector after the exhaust would be added to the extract acquired from the system clean out. Once the exhaust was complete, the collector, needle valve, and all tubing between the extractor and collectors were rinsed with hexane and methanol and collected in a rotor-evaporator ready flask.

Also the algae was removed from the extractor. The samples were then subjected to the same preparation and analysis as the samples of the Equilibrium Study.

The analysis applied to the extract from the Flow Study was the same as applied to the Equilibrium Study. Each fraction collected had the rinse solvent removed by rotor-evaporator. With the "dry" extract samples a gravimetric, TLC, and GC analysis would be conducted. The gravimetric and GC analysis were conducted the same as in the Pilot Study and Equilibrium Study. The TLC analysis was conducted in the same manner as the Equilibrium Study with the standard included. These analysis techniques provided the necessary data to investigate $SCCO_2$ extraction.

Results and Discussion

There were a total of six flow runs conducted for this study. The test conditions, CO_2 flow rate, and final extract mass are reported in Table 5. As can be seen, the CO_2 flow rate had a large amount of variation during the extractions. This is due to the solidification of the CO_2 inside the needle valve from rapid expansion. In an attempt to alleviate this problem, the needle valve was wrapped in heating tape and header tape, which helped reduce the severity of the blockages, but did not eliminate them. The net result is reflected in Table 5 with the flow of CO_2 varying greatly. For each run the pressure and temperature were within a standard deviation of the target value, with the exception of FR-5. The extraction temperature of FR-5 was lower than the target temperature of 100°C. This short coming is attributed to the combination of two factors. One being the vessel heater's set point has a maximum of 100°C. The second factor came from the lower pressure resulting in less heat of compression as the CO_2 was

pressurized. This resulted in less heat being delivered to the CO_2 which the vessel heaters could not compensate for.

Table 5. Condition of extractions for the Flow Study. The values for pressure, temperature, and flow rate are given as the mean ± standard deviation. The Total Extract is the total mass of the extract for each fraction + the extract found residual in the system. The Total Extract/ Sample Mass is the calculated value + the calculated value of the residual extract remaining in the system after extraction.

Run Name	Pressure (bar)	Temperature (°C)	CO_2 Flow Rate (scfh)	Total Extract (g)	Total Extract (g) /Sample Mass (g)
FR-1	400 ± 7.5	100 ± 6.7	36 ± 6.5	8.09 + 0.292	0.097 + 0.0035
FR-2	400 ± 4.5	83 ± 9.7	33 ± 8.9	9.88 + 0.650	0.12 + 0.0077
FR-3	200 ± 6.4	46 ± 2.5	33 ± 15	5.97 + 0.675	0.072 + 0.0081
FR-4	400 ± 5.7	38 ± 2.9	36 ± 13	6.11 + 0.420	0.073 + 0.0050
FR-5	100 ± 1.2	92 ± 3.9	44 ± 3.0	0.020 + 0.112	0.0002 + 0.001
FR-6	100 ± 1.5	41 ± 0.8	36 ± 8.8	0.343 + 0.111	0.004 + 0.001

As shown in Table 5, the run with the highest amount of extract recovered was FR-2. This run was at the highest pressure, but not the highest temperature. This may relate to the notion discussed in the literature review that the temperature has a negative effect on the extraction up to a point, then beyond that point temperature would have a positive effect on the extraction. The literature suggests this point is 400 bar or less [20, 36, 39, 44, 46], but the results from the FR-1 and FR-2 suggest that it might be at an even higher pressure. Interestingly, run FR-4 was at the same pressure as FR-2 but a lower temperature, and FR-4 had less extract recovered. This would suggest that the break point suggested in the literature may be more complex than a simple pressure and temperature, or even interdependence between the two parameters.

A more specific approach to the understanding the extract mass and the extraction conditions is through the density of the CO_2. As the literature states the density of the CO_2 correlates to the solvating power of the CO_2 [14, 35, 41, 44, 45]. The flow run that

corresponds to the most amount of extract is FR-2; however the CO_2 conditions for FR-2 do not correspond to the highest density. This is very interesting as some literature claims there exists a correlation between density and solvating power of $SCCO_2$, which would relate to the extraction rate and final extract mass. In fact most of the results presented here contradict that notion. FR-2 and FR-3 were conducted at the same CO_2 density and yet had a significant difference in the mass of total extract. Also, FR-4 had a similar amount of total extract recovered as FR-3, and these runs had different pressures and density with only the temperature relatively similar. Admittedly there is a general trend that lower densities, such as FR-5 and FR-6, yield less total extract than higher densities, like FR-2 and FR-4. However, based on the data presented here, density alone is not an adequate parameter to correlate to solvating power.

As with the Equilibrium Study, the gravimetric analysis yielded an extraction curve. Figure 11 shows the extraction curve for all of the flow runs. The graph provides more information than what was shown in Table 5. As shown before, FR-2 had the highest amount of total extract, but it is now possible to see that the last few fractions added very little to the total collected extract mass recovered from the run. This trend is apparent in most of the runs, with the last few fractions only adding a small amount of mass to the total extract mass. This is in agreement with the literature for the shape of the extraction curve for $SCCO_2$ extraction [16, 20, 31-33, 35-37, 40, 41, 43, 45, 48, 55]. From the data in Figure 11 it could be suggested that runs FR-1, FR-2, FR-4, and FR-5 have reached a plateau. It is unclear from the graph if FR-3 and FR-6 would continue to increase if more time were given for the extraction. The most interesting piece of information is the fact that the plateaus, for the runs exhibiting them, all ended at

different values. This is in agreement with some of the literature stating that the extraction conditions would not only affect the extraction rate but also the total possible amount of extract [35, 37, 40, 43, 55].

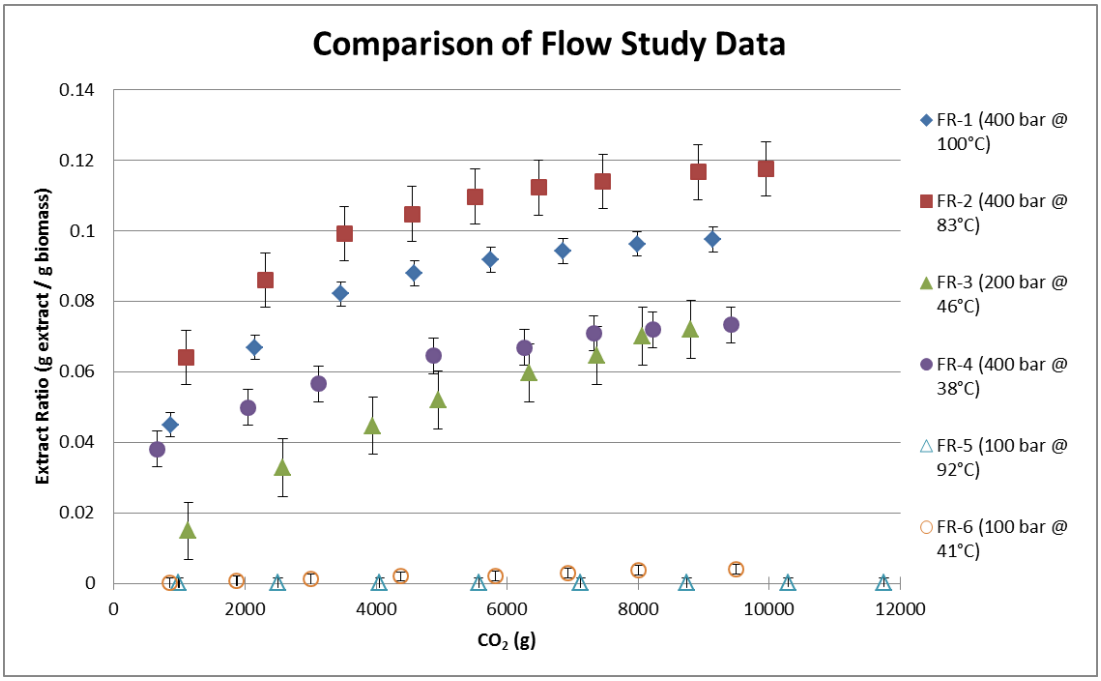

Figure 11. Graph showing the extraction curve for each of the flow runs. The vertical error bars represent the amount of extract found in the system after the extraction. The legend shows the mean value of the extraction conditions.

The results from the gravimetric analysis for the Flow Study extractions were used as a comparison to the previous studies. First, comparing the final extract weight of the Flow Study to the Pilot Study yielded some discrepancies. Specifically PR-6 and FR-5 had very similar extraction conditions, but PR-6 yielded 0.513g of extract where FR-5 yielded 0.132g of extract. The difference could be attributed to the residual extract and algae fines that were a problem with the Pilot Study configuration. This may have resulted in the pilot run producing an apparently larger extract amount. Another discrepancy is of PR-1 and FR-6. These two runs were conducted at very similar conditions; however the pilot run yielded about 3g of extract and the flow run yielded

only 0.43g of extract. Seeing how PR-1 was the first extraction conducted for the Pilot Study configuration, it would in error to attribute the total extract mass difference to any residual extract or algae fines that were deposited in the separators during the extraction. Both PR-1 and FR-6 had a continuous flow of CO_2, but the flow rate of PR-1 could not be measured. Also the duration of FR-6 was much longer than PR-1, but still resulted in a lower mass of total extract. The discrepancy between PR-1 and FR-6 has not been resolved. The pilot runs PR-3, PR-4, and PR-5 were all conducted at the same pressure but different temperature. These runs yielded a range of extract mass of 6.839g to 9.001g as shown in Table 2. These extract masses are similar to the extract masses obtained from FR-1, FR-3, and FR-4 as shown in Table 5. Interestingly, the pressure and temperature of the flow runs were significantly different from the pilot runs yet still yielded a similar extract mass. The final comparison between the Flow Study and previous studies is between FR-1 and ER-1. Figure 12 shows the extraction curve for each of the two runs on the same plot. The most striking result from this comparison is that even though both were conducted at the same conditions they yielded very different amounts of extract. The equilibrium run had about 50% more extract collected than the flow study run. One would expect to have a difference in the amount of extract collected between an equilibrium and flow arrangement as the flow configuration would have a mass transfer resistance whereas the equilibrium configuration would not. What could be expected is for the flow run to eventually reach the same total extract amount as the equilibrium run given enough time. The plot in Figure 12 shows that FR-1 appears to plateau which would mean the flow configuration could not yield the same amount of extract as the equilibrium configuration no matter how much time was given. This

discrepancy could be due to a couple of possibilities. The first being the membranes and cell walls of the algae in the equilibrium configuration were compromised since they were at temperature much longer than the algae sample in the flow configuration. The other possibility is the extracted lipid laden CO_2, of the equilibrium configuration, will extract more or different lipid compounds than the relatively neat CO_2 of the flow configuration. The exact cause of the difference in plateau amounts between the equilibrium and flow configurations was not determined.

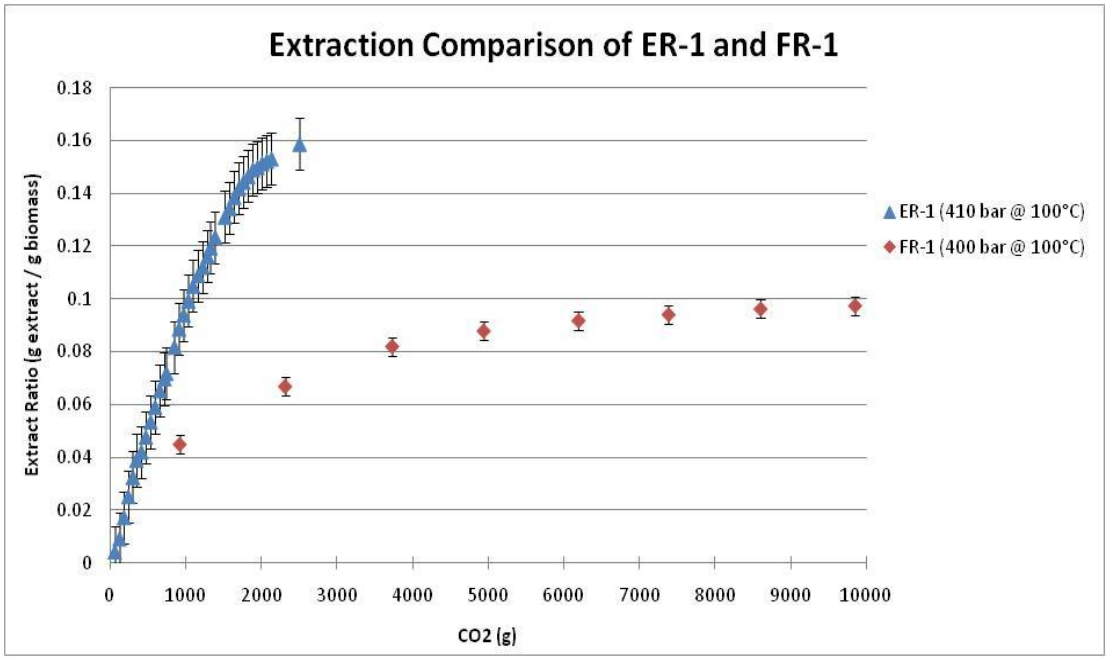

Figure 12. Graph showing the extraction curve for ER-1 and FR-1. The vertical error bars represent the amount of extract found in the system after the extraction. The conditions shown in the legend are the mean values.

The second analysis of the extract collected in the Flow Study was TLC. The TLC plates were prepared the same as in the Equilibrium Study including the standard. The exception is with FR-5 and FR-6, since these two runs yielded a very small amount of extract. The extract was more of a thin film on the inside of the rotor-evaporator ready flasks instead of a small pool as with the other runs. This proved to be difficult to take a

sample for TLC, and later GC. In order to pull a sample for TLC the two extracts were suspended in chloroform : methanol (2:1) by adding the solvent mixture directly to the rotor-evaporator ready flask. The solution was then treated as the sample for analysis and placed directly on the TLC plate. The first four flow runs did not require this suspension.

A photo of each TLC plate from the flow runs is shown in Figure 13. The spots on the plate were intentionally oversaturated since the TLC analysis is used only qualitatively for this research. As can be seen that every run has a dominant trend that nonpolar compounds were selectively extracted, although a few runs had some polars extracted as well. The plates for runs FR-1, FR-3, and FR-4 showed that only nonpolar compounds were extracted, which is in agreement with the results from the Pilot and Equilibrium Studies. The plates for runs FR-3, FR-5, and FR-6 showed a small amount of polar compounds. The polar spots for FR-2 appear only in the last three fractions. Recalling that FR-2 was the extraction with the most amount of extract collected, perhaps the extraction progressed so far that the extraction began to pull from the low polarity pool of compounds. This hypothesis could not explain the polar spots in FR-5 and FR-6, but there are a few possibilities to explain these polar spots. First, since these are the only two extractions at the low pressure (100 bar), perhaps these polar compounds are extractable only at the low pressure conditions for $SCCO_2$. Another possibility is from the method that the samples were handled after gravimetric analysis. Adding chloroform : methanol solution to the rotor-evaporator ready flask could have suspended not just the extract film, but also a contaminant that was on the inside of the flask. All the extract samples collected in the studies, with the exception of FR-5 and FR-6, were able to be directly pipetted out of the flask. This could be why a possible contaminant was not seen

in any of the other TLC analyses. A final possibility is that the few extracted polar compounds formed a precipitate in the extract and sank to the bottom of the flask. The precipitate would not have been pulled with the pipette in FR-1, FR-2, FR-3, and FR-4. However in FR-5 and FR-6 having the chloroform : methanol solution may have dissolved the precipitate, or by addition of the solution the precipitate was stirred up and thus pulled out with the pipette.

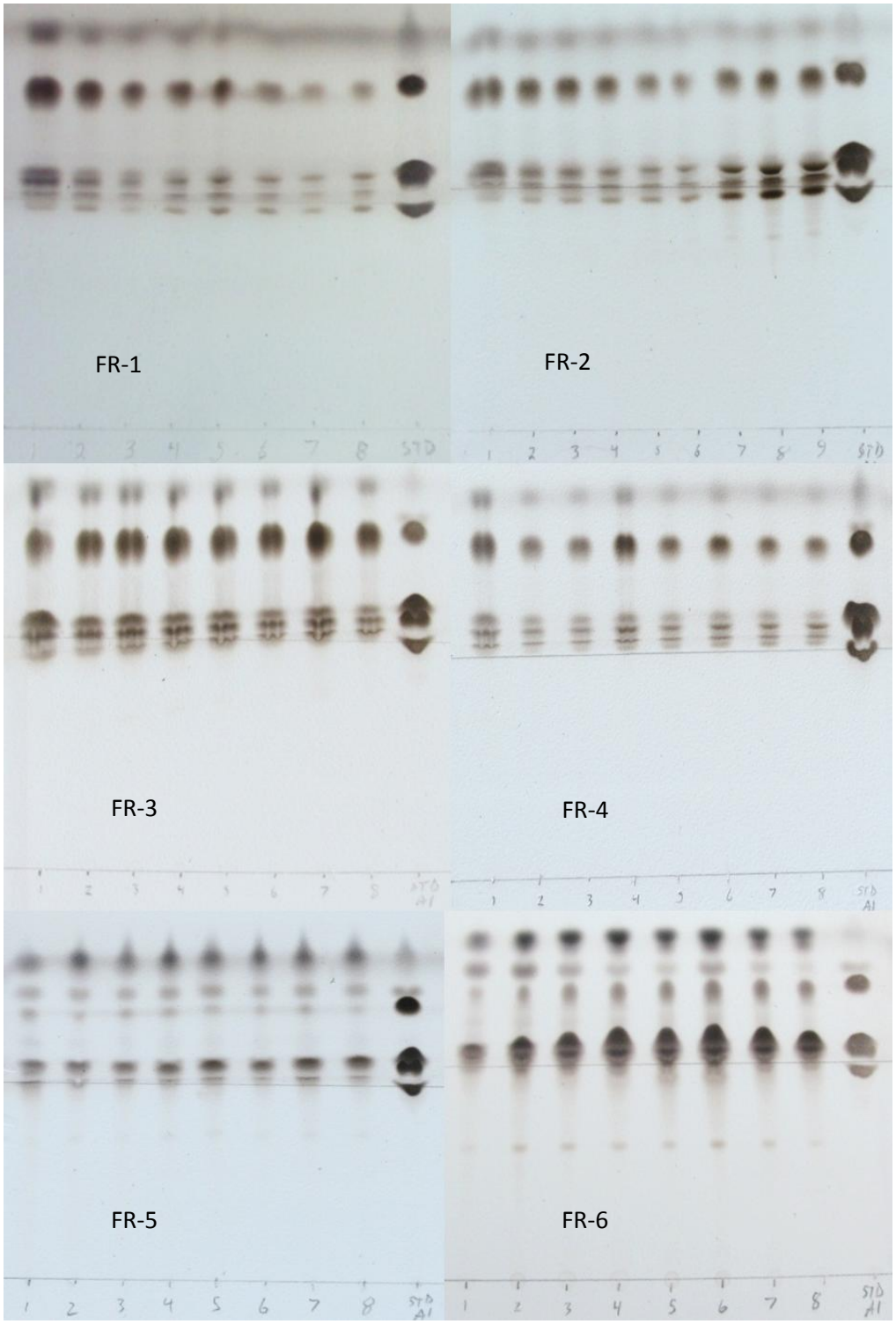

Figure 13. Photos of TLC plates for the Flow Study. The numbers correspond to the fraction number of the run. The last lane on each plate is the standard.

The final analysis of the extract obtained in the Flow Study was GC. The GC analysis was conducted the same as in the Pilot Study with a triplicate of the samples analyzed. The samples from FR-5 and FR-6 were submitted to GC in the same chloroform : methanol (2:1) solution that was described in the TLC analysis. Having the GC samples in solution resulted in the inability to measure the mass of the extract submitted to GC. This means that the GC results cannot be used to calculate the percent of mass transesterified. To keep consistency between data, all GC results were handled the same as in the Equilibrium Study in determining the relative amounts of saturation and unsaturation. This method was to directly apply the individual FAMEs percentage to the mass of extract for each fraction. This results in an assumption of 100% conversion of fraction extract mass into FAMEs. Although this may not be accurate it was the best way to ensure consistency between the $SCCO_2$ extract analyses. The GC analysis provided a lot of data so, for clarity, the GC results for each of the flow runs will be presented and discussed individually.

The GC results from the flow run FR-1 are shown in Figure 14. The plots show the relative percentage of each fatty acid chain, measured as FAMEs on the GC. Keeping in mind that Figure 14 shows only the relative values for the measured FAMEs, Figure 15 shows the actual total extract collected for each fraction. This flow run, FR-1, was at the same conditions as the equilibrium run, ER-1. The general trends are similar between the two runs; there was a decrease of 16:0 and 16:1 with a corresponding increase in 20:5. Even though the relative value of the 20:5 chain increases, the absolute amount of extract decreases throughout the extraction as shown in Figure 15. The GC data is also presented as a table by levels of saturation and unsaturation in Table 6. It is interesting to note that

the extract for the FR-1 conditions became increasingly more unsaturated throughout the extraction. The increase in unsaturation is also due to the poly-unsaturated fatty acid containing compounds, since the mono-unsaturated relative value is also decreasing over the course of the extraction.

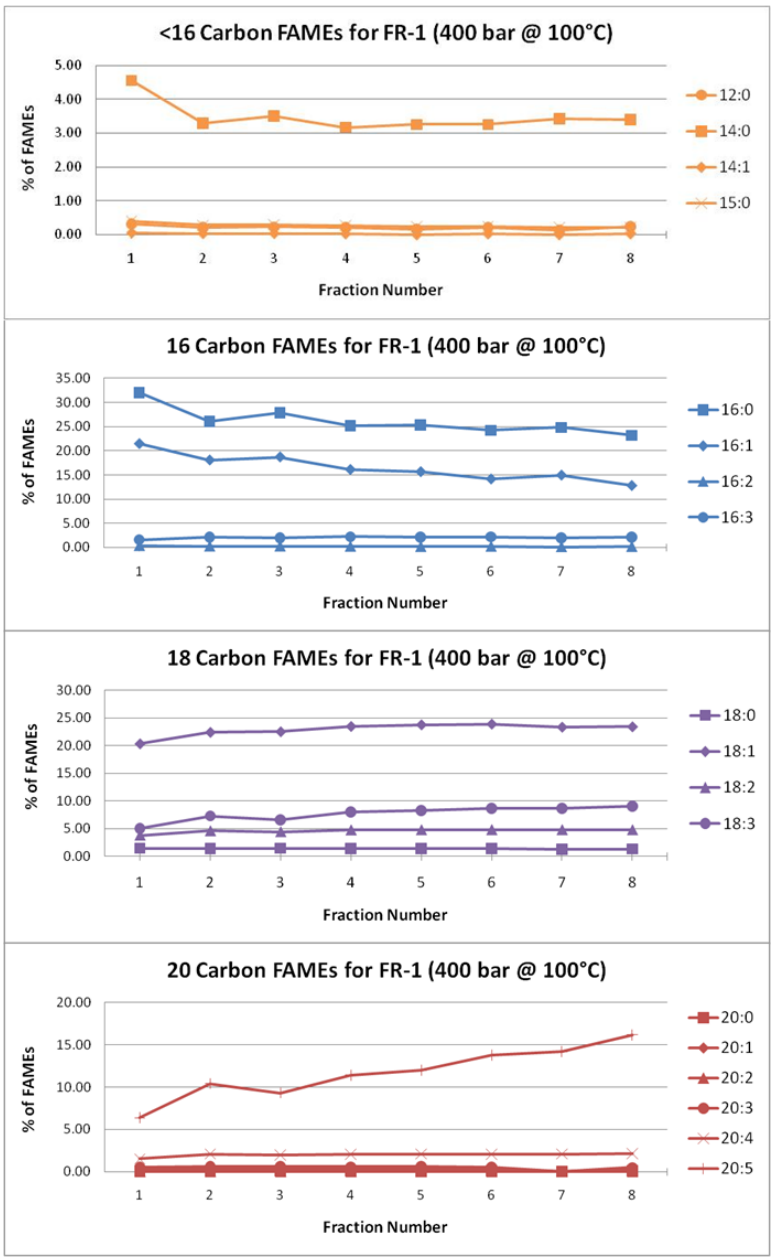

Figure 14. Lipid profile measured as FAMEs on GC of FR-1 over the course of the extraction. The following FAMEs are zero value throughout extraction: 20:2, 24:0, and 24:1. The 22 carbon FAMEs chart has been omitted since all values were 0%, with the exception of 22:2 which was 0.5%. The values shown on the plots are the mean values with the vertical error bars as the standard deviation.

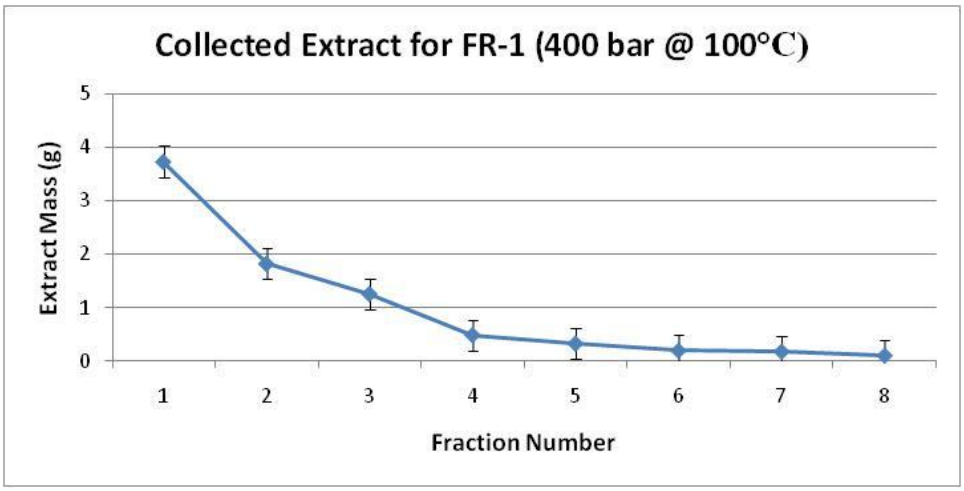

Figure 15. Mass of extract collected for FR-1. The values shown on the plot are the measured mass of each fraction with the vertical error bars representing the amount of extract found in the system after extraction.

Table 6. Relative amounts of saturation and unsaturation for FR-1. The heading indicates the total extract amount and each individual fraction amount. The values were calculated from the mean value of the FAMEs as measured with GC.

	Total	1	2	3	4	5	6	7	8
%Saturated	34.86	38.77	31.31	33.40	30.31	30.27	29.36	29.86	28.43
%Unsaturated	65.14	61.23	68.69	66.60	69.69	69.74	70.64	70.14	71.57
%Mono-Unsaturated	41.23	42.04	40.80	41.55	39.96	39.71	38.45	38.31	36.61
%Poly-Unsaturated	23.91	19.19	27.89	25.06	29.73	30.03	32.19	31.83	34.96
%Saturated / %Unsaturated	0.535	0.633	0.456	0.501	0.435	0.434	0.416	0.426	0.397

The next flow run, FR-2, was conducted at the same pressure as FR-1, but a slightly lower temperature. The GC results are shown as plots in Figure 16. As can be seen, the same trends that dominated FR-1 are present in FR-2. The relative values of the 16:0 and 16:1 chains decrease over the course of the extraction and the 20:5 increases. However the absolute amount of the extract has decreased as shown in Figure 17. The slight difference between FR-1 and FR-2 is that some of the chains appear more consistent throughout the extraction. For example, 18:3 does not increase as much in FR-

2 as it did for the previous run. Table 7 shows the relative saturation and unsaturation for FR-2. The same trend of increasing unsaturation throughout the extraction is seen. Interestingly, FR-2 appears to start at a lower relative amount of saturation than the previous extractions. As with FR-1, the increase in unsaturation over the course of the extraction is due to the poly-unsaturated compounds since the mono-unsaturated compounds decrease throughout the extraction.

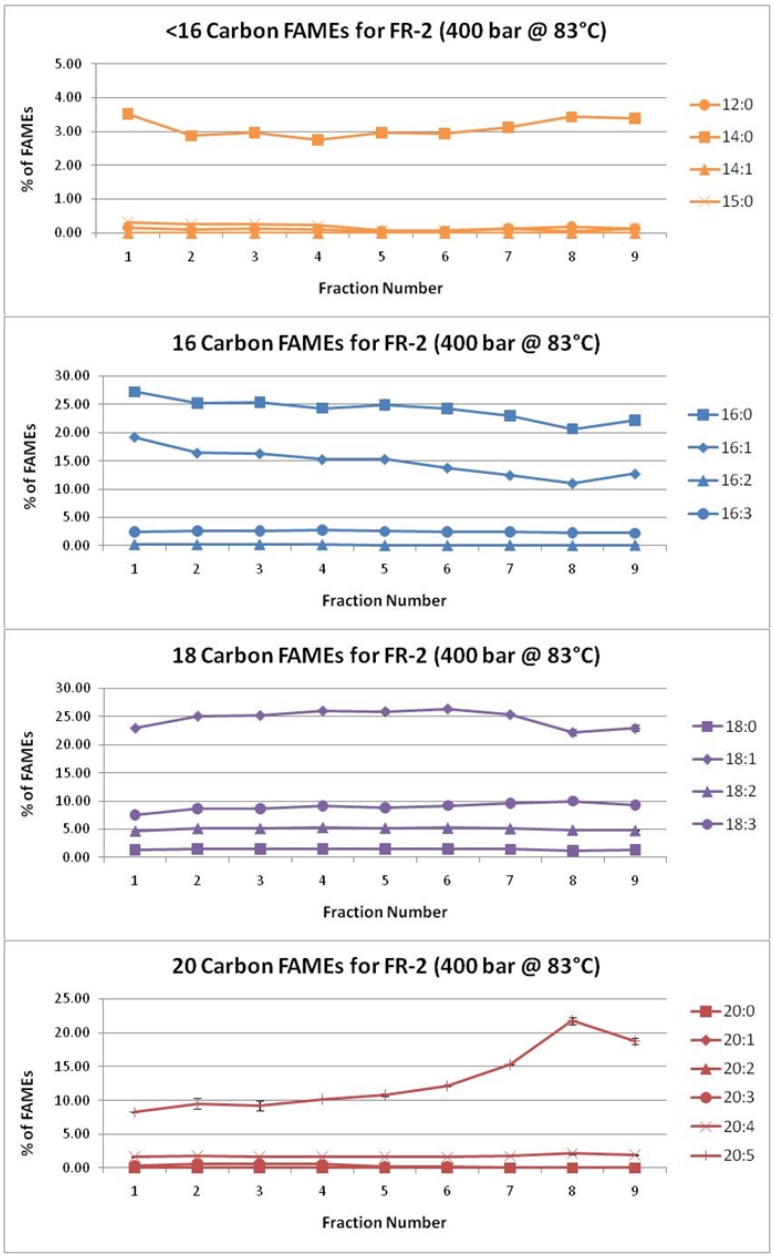

Figure 16. Lipid profile measured as FAMEs on GC of FR-2 over the course of the extraction. The following FAMEs are zero value throughout extraction: 14:1, 20:0, 20:2, 24:0, and 24:1. The 22 carbon FAMEs chart has been omitted since all values were 0%. The values on the plots are the mean value with the vertical error bars representing standard deviation.

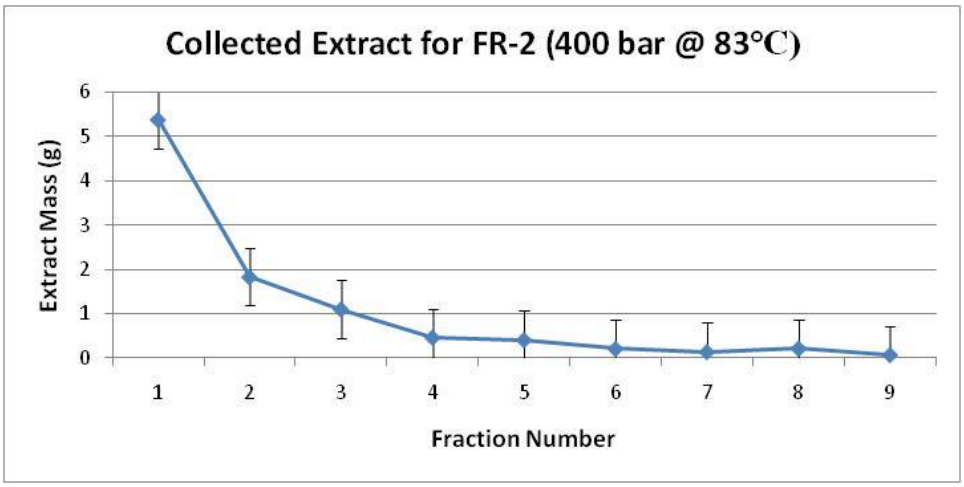

Figure 17. Mass of extract collected for FR-2. The values shown on the plot are the measured mass of each fraction with the vertical error bars representing the amount of extract found in the system after extraction.

Table 7. Relative amounts of saturation and unsaturation for FR-2. The heading indicates the total amount and each individual fraction amount. The values were calculated from the mean value of the FAMEs as measured with GC.

	Total	1	2	3	4	5	6	7	8	9
%Saturated	31.16	32.59	28.90	30.18	28.88	29.53	28.84	27.81	25.57	27.16
%Unsaturated	68.84	67.42	70.10	69.82	71.13	70.47	71.16	72.19	74.43	72.84
%Mono-Unsaturated	41.57	42.21	41.52	41.66	41.33	41.21	40.18	37.83	33.27	35.66
%Poly-Unsaturated	27.27	25.21	28.58	28.16	29.79	29.26	30.98	34.36	41.17	37.19
%Saturated / %Unsaturated	0.453	0.483	0.426	0.432	0.406	0.419	0.405	0.385	0.343	0.373

The next flow run, FR-3, was conducted at a lower pressure and temperature than the previous flow runs, but at the same density of CO_2 as FR-2. The GC results are shown in Figure 18. The relative value of the chains 16:0 and 16:1 decrease, as seen before, along with 20:5 increasing. However, at these conditions the increase of 20:5 is not as pronounced as FR-1 or FR-2. Also the relative increase of 18:1 is much more pronounced for these conditions than the previous flow runs. To put the trends in perspective Figure 19 shows the absolute value of the extract over the course of the

extraction. Interestingly, Figure 19 shows that the FR-3 extraction did not have a smooth inverse extraction curve as FR-1 and FR-2 did, but the uncertainty for FR-3 is relatively larger than the previous flow runs. Table 8 shows the relative saturation and unsaturation of the extract from FR-3. The same trend of increasing unsaturation throughout the extraction is seen here as well. Interestingly, FR-3 is the first extraction that has both the poly-unsaturated and mono-unsaturated relative amounts increasing throughout the extraction. Also FR-3 appears to be more saturated throughout the extraction than FR-2 which was at the same CO_2 density.

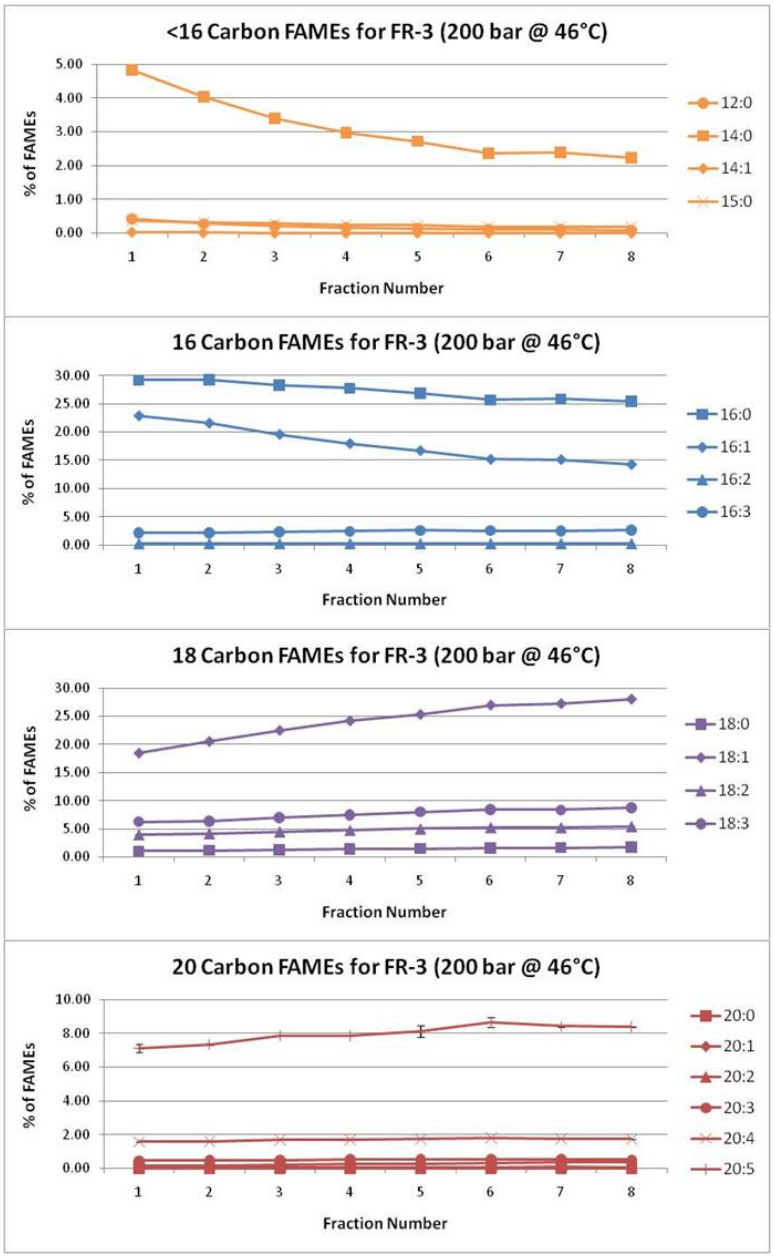

Figure 18. Lipid profile measured as FAMEs on GC of FR-3 over the course of the extraction. The following FAMEs are zero value throughout extraction: 24:1. The 22 carbon FAMEs chart has been omitted since all values were 0%, except 22:0 and 22:1 which were <0.5%. The values on the plots are the mean value with the vertical error bars representing standard deviation.

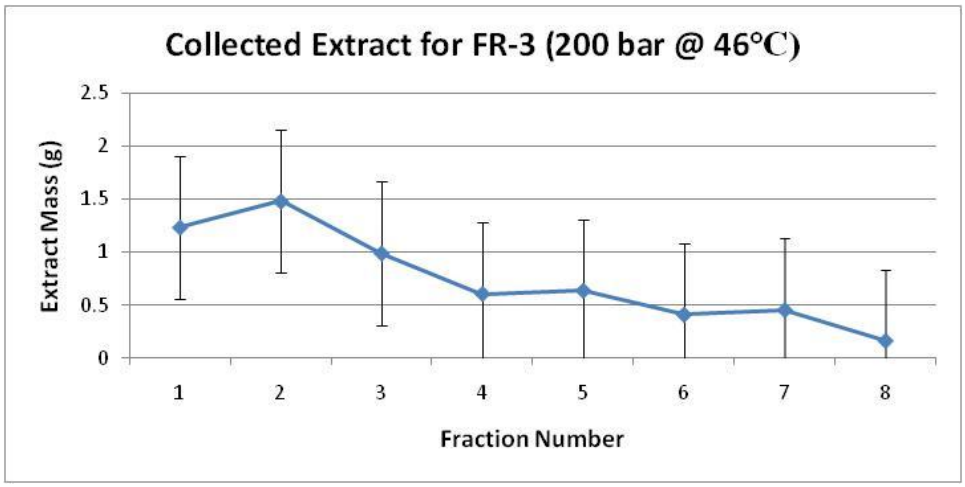

Figure 19. Mass of extract collected for FR-3. The values shown on the plot are the measured mass of each fraction with the vertical error bars representing the amount of extract found in the system after extraction.

Table 8. Relative amounts of saturation and unsaturation for FR-3. The heading indicates the total amount and each individual fraction amount. The values were calculated from the mean value of the FAMEs as measured with GC.

	Total	1	2	3	4	5	6	7	8
%Saturated	33.49	35.91	35.05	33.48	32.61	31.50	30.03	30.26	26.70
%Unsaturated	66.51	64.09	64.95	66.52	67.39	68.50	69.97	69.74	70.30
%Mono-Unsaturated	42.28	41.98	42.28	42.27	42.38	42.30	42.47	42.71	42.65
%Poly-Unsaturated	24.22	22.11	22.66	24.25	25.01	26.20	27.50	27.03	27.65
%Saturated / %Unsaturated	0.504	0.560	0.540	0.503	0.484	0.460	0.429	0.434	0.422

The next flow run, FR-4, was at the same pressure as FR-1 and FR-2, but at a lower temperature. The plots in Figure 20 show the relative amounts of the fatty acid chains over the course of FR-4. As with the other flow runs the chains 16:0 and 16:1 decrease over the course of the extraction. FR-4 differs in that the chains 20:4 and 20:5 appear not to increase, but actually slightly decrease by the end of the extraction. For this flow run 18:1, 18:2, and 18:3 all appear to increase over the course of the extraction. Keep in mind that the absolute yield of extract decreases throughout the extraction as

shown in Figure 21. From Table 9 it is apparent that FR-4 follows the same trend as FR-1 and FR-2 with the relative amount of unsaturation increasing throughout the extraction with the poly-unsaturates being the reason for the increase.

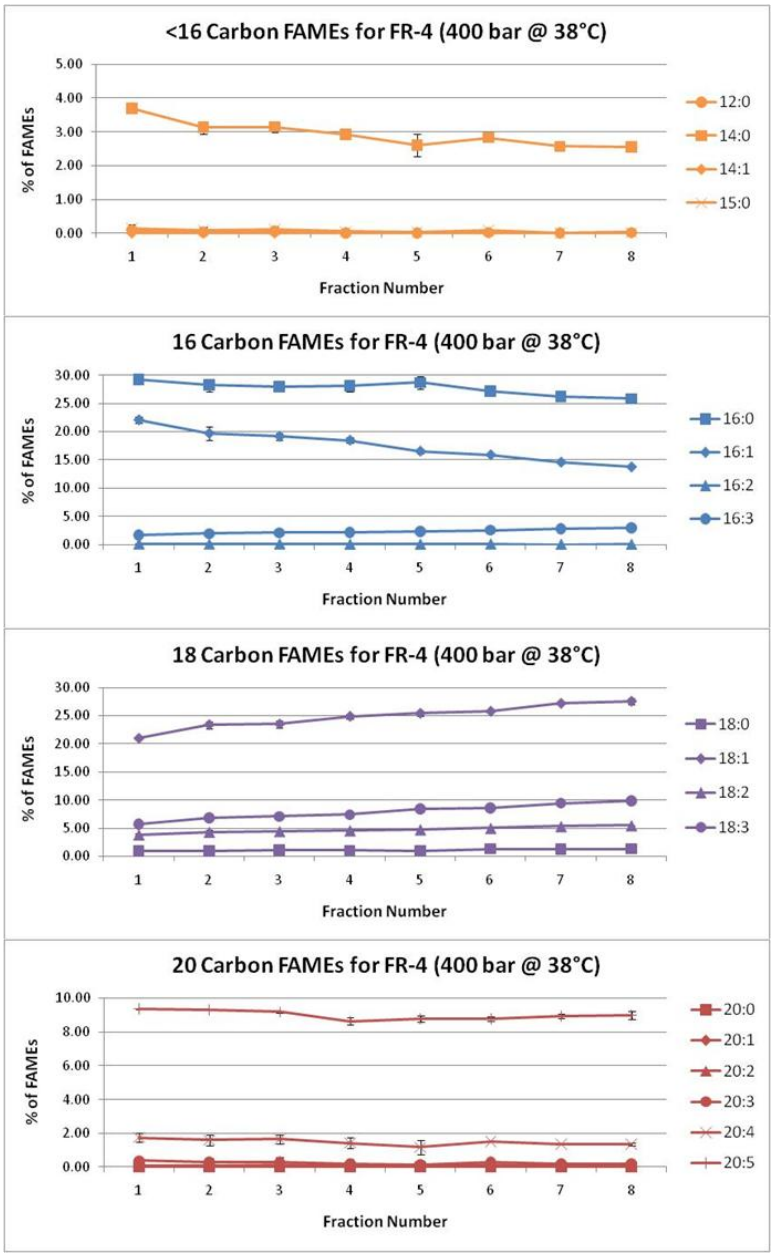

Figure 20. Lipid profile measured as FAMEs on GC of FR-4 over the course of the extraction. The following FAMEs are zero value throughout extraction: 14:1, 20:0, 20:2, 24:0, and 24:1. The 22 carbon FAMEs chart has been omitted since all values were 0%. The values on the plots are the mean value with the vertical error bars representing standard deviation.

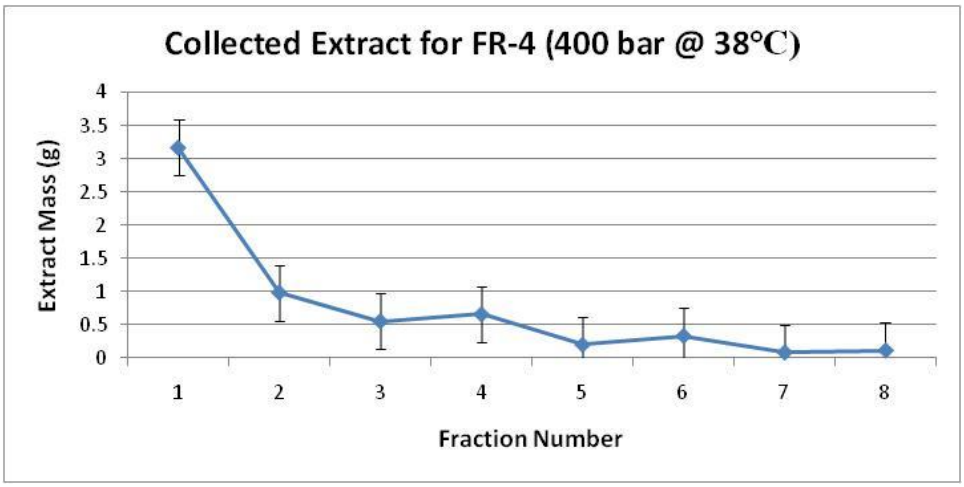

Figure 21. Mass of extract collected for FR-4. The values shown on the plot are the measured mass of each fraction with the vertical error bars representing the amount of extract found in the system after extraction.

Table 9. Relative amounts of saturation and unsaturation for FR-4. The heading indicates the total amount and each individual fraction amount. The values were calculated from the mean value of the FAMEs as measured with GC.

	Total	1	2	3	4	5	6	7	8
%Saturated	33.14	34.13	32.46	32.39	32.16	32.33	31.43	30.09	29.76
%Unsaturated	66.86	65.87	67.54	67.61	67.84	67.67	68.57	69.91	70.24
%Mono-Unsaturated	42.96	43.11	43.16	42.74	43.42	41.99	41.79	41.81	41.38
%Poly-Unsaturated	23.90	22.76	24.38	24.87	24.43	25.68	26.78	28.11	28.86
%Saturated / %Unsaturated	0.496	0.518	0.481	0.479	0.474	0.478	0.458	0.430	0.424

The flow run FR-5 was the first extraction for the Flow Study at a low pressure. As stated before this run is one of two that had the extract suspended in chloroform : methanol to be analyzed. This meant that the transesterification reactants were applied to the chloroform : methanol solution directly, as the solution was treated as extract. The results from the GC proved to be difficult to analyze for FR-5. Figure 22 shows the plots obtained from the GC analysis. These results differ greatly from the previous flow runs. There is an apparent “saw tooth” pattern in the data that only affects some of the chains,

but not others. Since a triplicate of sample was analyzed by GC for each fraction, the mean and standard deviation is shown in Figure 22. However the standard deviation is very small compared to the "saw tooth" pattern which would suggest this pattern can't be explained by uncertainty. The pattern may arise from the fact that so little extract was collected in each fraction. Any non-uniformity in the rate of extraction of different compounds would have a greater impact on the FAMEs profile than other flow runs with much larger fraction masses. Figure 23 shows how the uncertainty is much larger than the collected extract for each fraction. This relatively large uncertainty could be attributed to a couple of possibilities. Perhaps the lower extraction conditions do not provide enough driving force to push the extract out of the tubing, or a point of saturation of extract in the tubing needs to be reached before the extract can be easily pass through the tubing. At these conditions there seems to be more diversity in the number of chains that are over 10% of the FAMEs. The chains 18:3, 20:2, 20:3, and 22:5 surpass 10% for many fractions. This is very interesting as these chains are much lower in the previous flow runs. Especially the 22 carbon FAMEs, which were zero or less than a percent of the total in previous runs these FAMEs. The "saw tooth" pattern makes it more difficult to suggest that the relative value of the chains 16:0 and 16:1 are decreasing throughout the extraction as with the previous flow runs. Also more similarly to FR-4 than the first few flow runs, the chains 20:4 and 20:5 appear to remain fairly constant throughout the extraction. The "saw tooth" pattern not only makes the plots in Figure 22 difficult to analyze but also the relative saturation and unsaturation amounts reported in Table 10. From the table, fractions 6 and 8 had a non-measureable amount of extract which causes the calculation for the table values to yield zero. From the table it can be seen that FR-5

had some of the most unsaturated extract. This could be due to the fact that there were considerably less mono-unsaturated lipids in this run compared to previous flow runs.

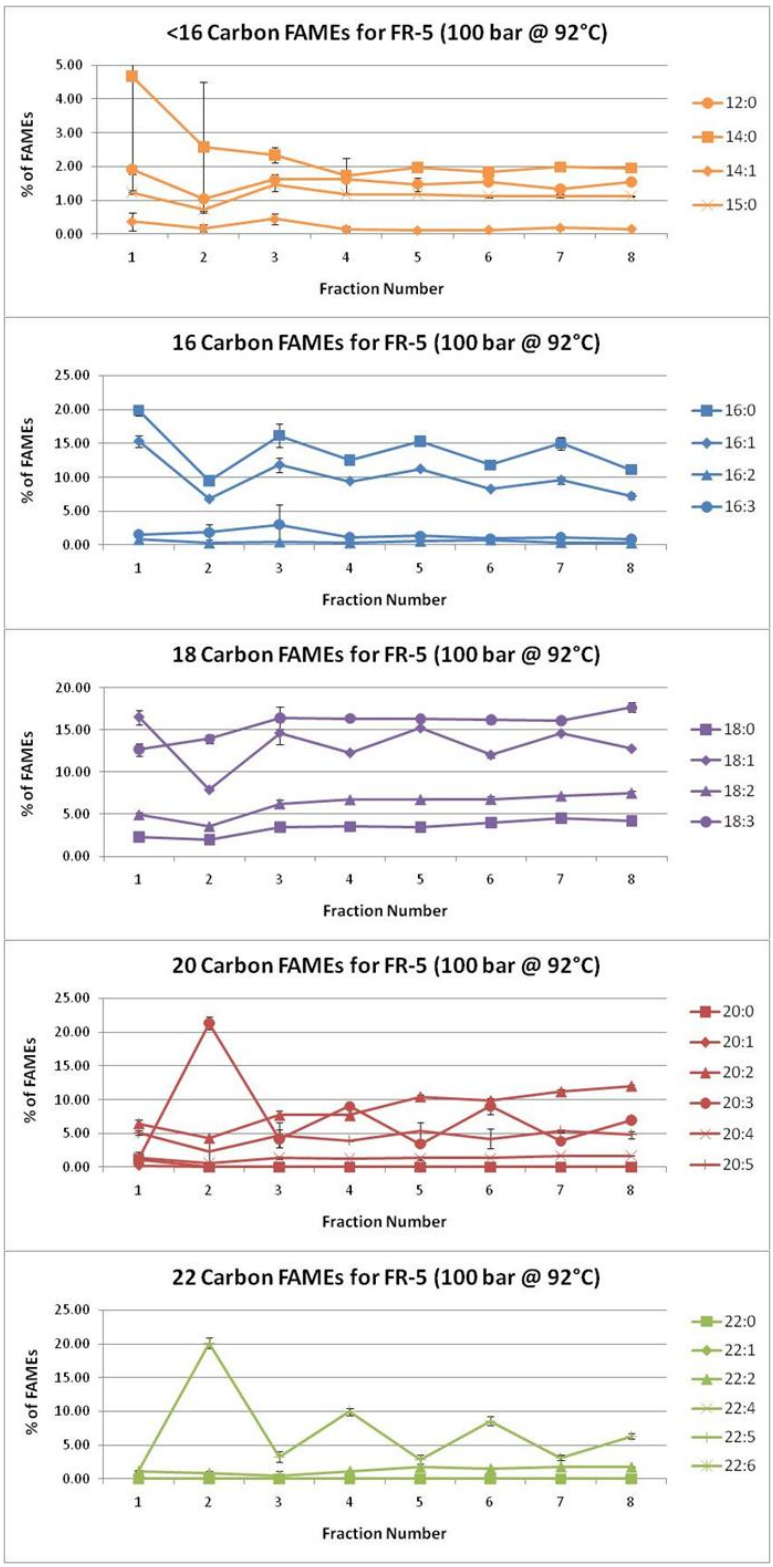

Figure 22. Lipid profile measured as FAMEs on GC of FR-5 over the course of the extraction. The following FAMEs are zero value throughout extraction: 22:0, 22:1, 22:4, 22:6, 24:0, and 24:1. The values on the plots are the mean value with the vertical error bars representing standard deviation.

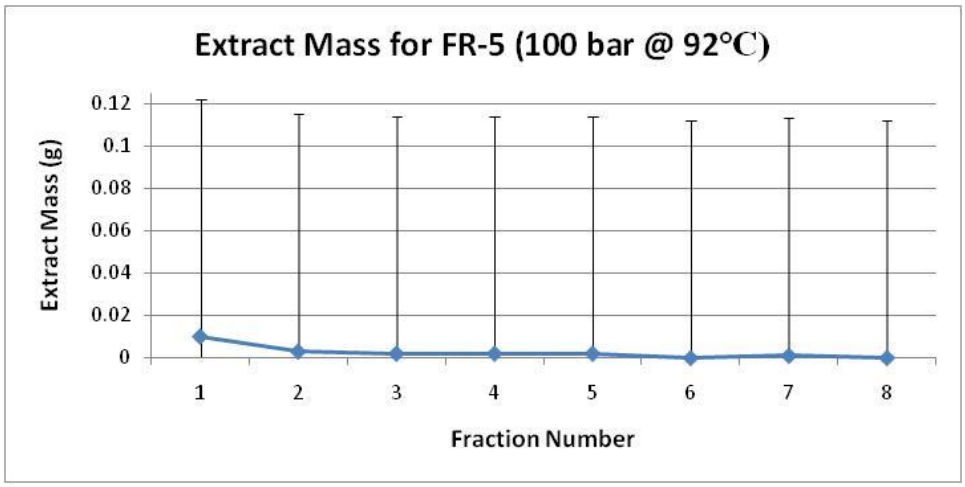

Figure 23. Mass of extract collected for FR-5. The values shown on the plot are the measured mass of each fraction with the vertical error bars representing the amount of extract found in the system after extraction.

Table 10. Relative amounts of saturation and unsaturation for FR-5. The heading indicates the total amount and each individual fraction amount. Fractions 6 and 8 did not have a measureable amount of extract. The values were calculated from the mean value of the FAMEs as measured with GC.

	Total	1	2	3	4	5	6	7	8
%Saturated	26.06	31.18	15.82	25.04	20.63	23.36	NA	23.97	NA
%Unsaturated	73.94	68.82	84.18	74.96	79.37	76.64	NA	76.03	NA
%Mono-Unsaturated	27.22	32.45	14.96	26.99	21.82	26.51	NA	24.30	NA
%Poly-Unsaturated	46.72	36.37	69.22	47.97	57.55	50.13	NA	51.73	NA
%Saturated / %Unsaturated	0.353	0.453	0.188	0.334	0.260	0.305	NA	0.315	NA

The last flow run, FR-6, was conducted at the mildest extraction conditions for this study. Similarly to FR-5, the extract had to be suspended in chloroform : methanol (2:1) to be handled. The results from the GC analysis are shown in Figure 24. FR-6 was very peculiar because it is the first and only of the flow runs that had the chains 14:0, 16:0, and 16:1 increasing over the course of the extraction. Another interesting trend is that of the 18 carbon chains. In the other flow runs the 18 carbon chains slightly increase or remain relatively consistent throughout the extraction, but for FR-6 the 18 carbon

chains appear to decrease or remain relatively consistent throughout the extraction. The next noteworthy point is the large crest of the chains 20:2 and 22:2. FR-5 is the only other flow run that had a large amount of either of these chains. This would suggest that the low pressure of FR-5 andFR-6 is selective towards the lipids that contain these chains. The chain 24:1 was not shown on the plot, but maintains a relative value of 1% for the entire extraction. This stands out since in all the previous runs the chain 24:1 had a zero value, less than 1%, or was only present in a few fractions before dropping to zero. Figure 25 shows the extract mass over the course of the extraction. Similarly to FR-3, the extract mass of FR-6 does not drop off as a smooth curve. The GC results were also used to compute the relative saturation and unsaturation amounts for FR-6 as shown in Table 11. The table shows that FR-6 is the only flow run that had an increasing amount of saturation over the course of the extraction. However, FR-6 also had the lowest amount of relative saturation to begin with. The table also shows how FR-6 had an increase in the relative amount of mono-unsaturation throughout the extraction with a corresponding decrease in the relative amount of poly-unsaturated lipids.

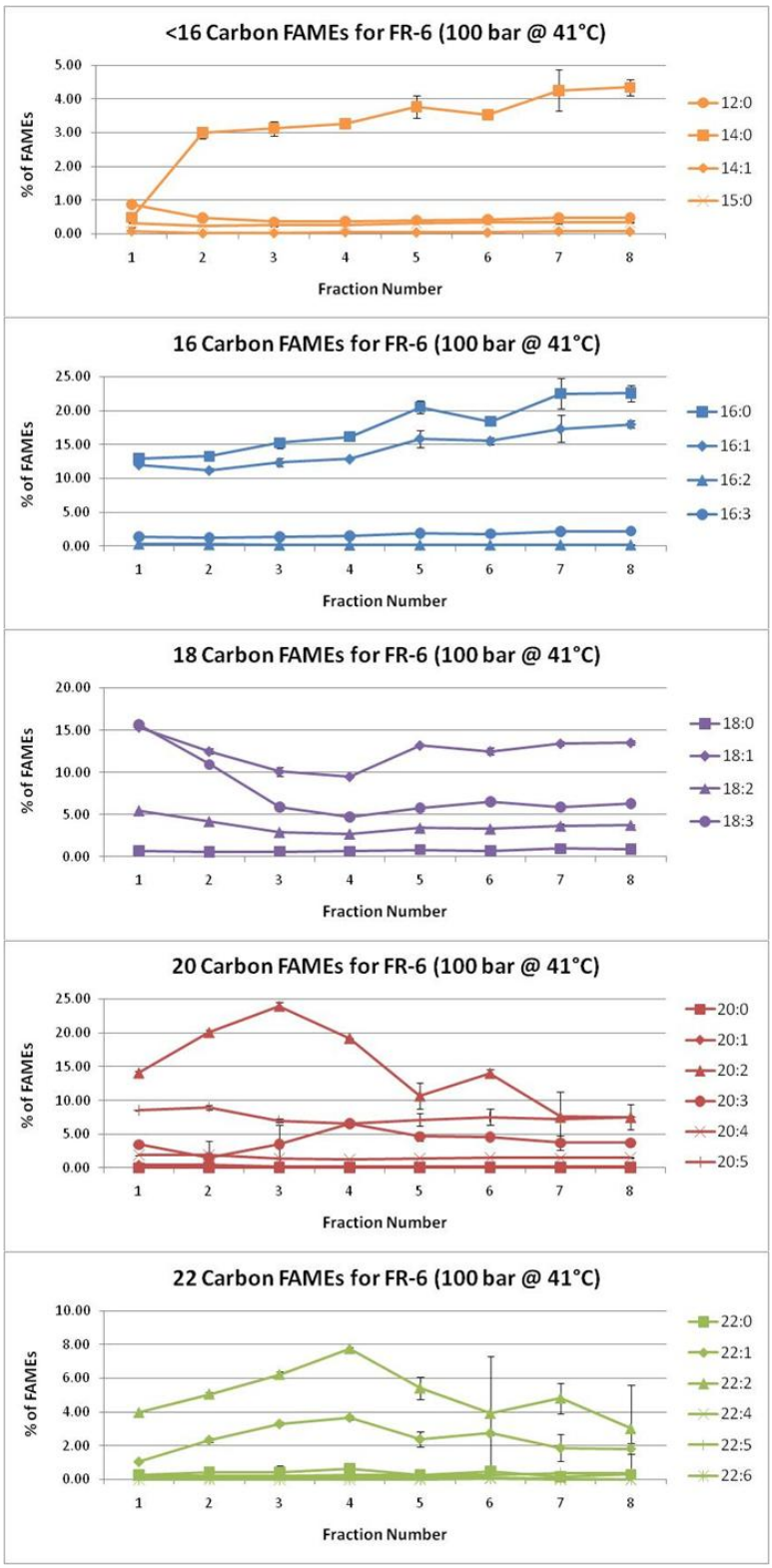

Figure 24. Lipid profile measured as FAMEs on GC of FR-6 over the course of the extraction. The following FAMEs are zero value throughout extraction: 24:0. The values on the plots are the mean value with the vertical error bars representing standard deviation.

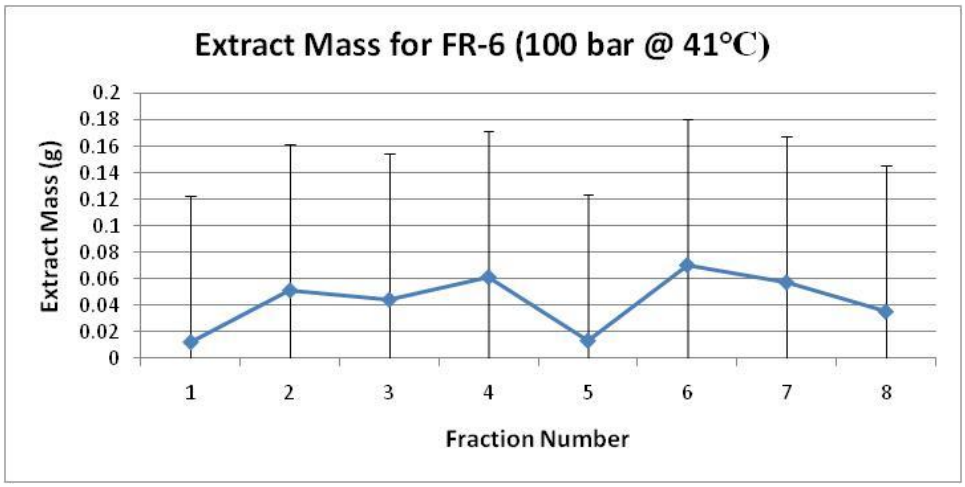

Figure 25. Mass of extract collected for FR-6. The values shown on the plot are the measured mass of each fraction with the vertical error bars representing the amount of extract found in the system after extraction.

Table 11. Relative amounts of saturation and unsaturation for FR-6. The heading indicates the total amount and each individual fraction amount. The values were calculated from the mean value of the FAMEs as measured with GC.

	Total	1	2	3	4	5	6	7	8
%Saturated	23.26	15.63	18.07	20.12	21.42	26.19	23.98	28.80	29.01
%Unsaturated	76.75	84.38	81.93	79.88	78.58	73.81	76.02	71.20	70.99
%Mono-Unsaturated	30.47	29.11	27.35	27.06	27.67	32.80	34.06	34.02	34.85
%Poly-Unsaturated	46.27	55.26	54.58	52.82	50.91	41.01	43.97	37.18	36.14
%Saturated / %Unsaturated	0.303	0.185	0.220	0.252	0.273	0.355	0.315	0.404	0.409

An interesting point should be made about the actual chromatograms from the $SCCO_2$ extractions FR-5 and FR-6. It is common for the chromatograms to have a number of peaks that do not correspond to a FAME in the standard, and as such will not be identified by the software of the GC machine. For the runs FR-5 and FR-6 there were about double the amount of unidentified peaks. Although this should not affect the GC's ability to identify the FAME peaks it did. To compensate for the lack of identification or misidentification of the peaks by the software a manual approach was taken in order to

use the GC data to identify the FAME peaks. This was accomplished with a printout of the standard's profile and a printout of the extract profile. These printouts were overlaid and compared on a light table. The extract peaks were determined by matching the standard's peak to the largest peak that was closest on the extract chromatogram for each individual FAME. Not having the software identify the peaks also means there is no conversion of the peak area to an actual FAME mass. This was resolved by determining the relative amounts of FAMEs by using their relative peak areas, instead of the software's calculated mass. By using the relative peak area the FAME profile and thus saturation and unsaturation could be determined. This manual identification provided a way for all the GC results to be compared but at the cost of consistency in data analysis.

With the data obtained from the Flow Study configuration, a rudimentary energy ratio can still be determined. This could provide some insight for future research that utilizes $SCCO_2$ for lipid extraction with the explicit intention of biofuels. The energy ratio was determined by the quotient of the energy in and energy out. The energy in is the energy put into the CO_2 and the energy out is based on the assumption that the extract has the same energy density as petroleum based diesel. The energy put into the CO_2 was determined by the mass of CO_2 measured for the extraction multiplied by the change of enthalpy from the CO_2 cylinder to extraction conditions [68, 69]. (The CO_2 cylinder conditions were assumed to be 51.7 bar at 20°C.) The energy of the extract was determined by the measured mass of extract multiplied by the energy density of diesel, which is about 44 MJ/kg [70-72]. The results of these energy calculations are shown in Table 12. An interesting point is that the flow run that yielded the most extract, FR-2, did not have the best energy ratio. The best energy ratio was for the single equilibrium run,

but focusing on the flow runs the best energy ratio is from FR-1. Even though the FR-1 run had the most severe conditions it had the smallest change in enthalpy from the cylinder conditions.

Table 12. Calculation results of the energy ratio for the Flow Study. The conditions shown are the average values for the conditions. The last row shows results from the equilibrium run for comparison. Ratio is the energy ratio and calculated as described in text above.

Run Name	Enthalpy Change (kJ/kg)	Mass of CO2 (kg)	Mass of Extract (g)	Ratio (Energy in / Energy out)
FR-1 (400 bar @ 100°C)	48.62	9.143	8.383	1.205
FR-2 (400 bar @ 83°C)	80.07	9.951	10.53	1.720
FR-3 (200 bar @ 46°C)	137.1	8.806	6.643	4.129
FR-4 (400 bar @ 38°C)	163.2	9.415	6.526	5.351
FR-5 (100 bar @ 92°C)	63.59	11.75	0.132	128.7
FR-6 (100 bar @ 41°C)	108.7	9.497	0.454	51.67
ER-1 (410 bar @ 100°C)	48.62	2.501	13.31	0.208

With the completion of the Flow Study it would be beneficial to this analysis and to other research if there were general trends related to the extraction conditions. It is difficult to make broad generalizations from the data collected in the Flow Study, but a few can be made. The first trend is that the high pressure points all yielded similar lipid profiles. The highest extract yield was not at the most severe conditions, but at the high pressure with a slightly less than highest temperature. Another trend is the extractions at low pressure resulted in a greater diversity of chains representing over 10% of the total FAMEs, along with a greater number of unknown peaks in the chromatograms. Also for the low pressure points, the TLC plates showed a polar spot for all fractions. The last trend is that of the conditions tested, the extractions that occurred at 200 bar or higher had an increasing amount of unsaturation over the course of the extraction. The knowledge of the relative amounts of saturation and unsaturation could be beneficial to future

research that would use $SCCO_2$ to extract lipids for biodiesel production, to see if there was an ideal split between saturation and unsaturation of lipids for biodiesel.

The Flow Study combined all the operational improvements presented by the Pilot Study and Equilibrium Study. However there are still more improvements that could be made to the Flow Study configuration. The biggest improvement would be to have an electronic gas flow totalizer or integrator that could record the CO_2 flow rate dynamically and report the total CO_2 used at any specified time interval. This would eliminate the large error associated with the current method of assuming a linear flow rate between times of recorded CO_2 flow. Another improvement would be to have an internally heated needle valve or regulator for the CO_2 depressurization. Currently the heating is applied externally to the needle valve which provides some benefit to reduce the CO_2 solidification from the rapid depressurization in the needle valve. However, having the heat source internally at the orifice would provide a much greater benefit to preventing blockage inside the needle valve. The final improvement would be for the handling of extract that is not able to be pipetted. Suspending the extract in a solution of chloroform : methanol could still be used, but then perhaps the solution should be rotor-evaporated dry once the solution was placed in the vial for transesterification. Although there is no known effect of chloroform on the transesterification reaction, it would be in the best interest of the research to remain consistent with all samples for analysis.

Conclusion

The Flow Study was the last set of experiments using $SCCO_2$ for extraction of lipids. This study maintained a constant flow of CO_2 through the algal sample. The

extraction condition that yielded the highest amount of extract was FR-2 at 400 ± 4.5 bar and 83 ± 9.7°C. These conditions also yielded the largest relative amount of the chain 20:5. At the low pressure, 100 bar, there was a much greater diversity of FAMEs over 10% of the total FAMEs collected. Also the low pressure conditions showed a polar spot on the TLC plates for all fractions collected. For all conditions tested above 100 bar there was an increase in the amount of unsaturation over the course of the extraction. The run FR-6, at a low extraction pressure and temperature had an increase in saturation throughout the extraction. Without the limitation of the available resources more concise data could be collected with an electronic flow meter and an internally heated needle valve.

IV. Conclusion and Future Work

Overall, there has been a lot of work done with this $SCCO_2$ apparatus. The progress made is as much operational improvements as it is scientific knowledge gain. Each study conducted increased the operational knowledge. Whether this knowledge was as simple as learning the order to open and close valves or when to turn on the vessel heaters. The studies also proved to be beneficial by learning from the deficiencies of each configuration. The Pilot Study was deficient with the lack of instrumentation to measure the CO_2 flow rate. The Equilibrium Study provided a concise measurement of the CO_2 amount, but at the cost of a practical experimental duration. Finally the Flow Study enabled a method for measuring the CO_2 flow rate within a practical experimental duration, but at the cost of preciseness of the measurement. As with much research, one of the largest limiting factors in addressing deficiencies was budget. For the work conducted in this thesis, the majority of improvements had to be made with what was available at the time as there was very little room in the budget for extra expenditures.

The Pilot Study and Equilibrium Study provided some insight into the specifics of using CO_2 to extract lipids from an algal sample, but the Flow Study provided the most knowledge. From the Pilot Study the conditions that yielded the most extract were 350 ± 7.4 bar at $50 \pm 12°C$ which yielded 9.0g. However, there was a concern that all of the Pilot Study extract masses were off due to a problem of residual material remaining in the system. The Equilibrium Study did not have a problem of residual material as the Pilot

Study had, and yielded the most amount of extract of 13.3g at the conditions of 410 ± 31 bar at 100 ± 0.1°C. It was not determined how much more extract could be recovered, but it was assumed to be very close to an exhaustive extraction. The Flow Study provided the most amount of data for $SCCO_2$ extraction of an algal sample. The highest yield from the Flow Study was 10.53g at the conditions of 400 ± 4.5 bar at 83 ± 9.7°C. Interestingly, this corresponded to the second best energy ratio of 1.720. The best energy ratio was 1.205 which came from the extraction at 400 ± 7.5 bar at 100 ± 6.7°C. All of the studies provided evidence that $SCCO_2$ is selective towards neutral compounds. The 100 bar extractions from the Flow Study showed polar compounds, but it was not verified if the compounds were from the extraction or a contaminant from handling. It was also found that in general the relative amount of unsaturation increased throughout the extraction, with the exception of the flow run at 100 ± 1.5 bar at 41 ± 0.8°C which had the unsaturation decrease over the course of the extraction. Even more data could be obtained with more developed analytical methods.

The research presented in this thesis was an exploratory endeavor into $SCCO_2$ extraction, leaving room for future work involving $SCCO_2$ extraction of an algal sample. Future experiments that may involve this apparatus could greatly benefit from having more modern electronic data collection systems. Such as a gas flow meter that can record the continuous CO_2 flow and then determine the total amount of CO_2 that passes by for a given interval. Another improvement would be to have a similar electronic collection for the temperature and pressure of the extractor. Although these may not vary as much as the CO_2 flow rate, having a modern data collection system would make the results more concise.

One aspect that would greatly enhance the quality of research obtained from this $SCCO_2$ extraction apparatus would be a strong emphasis on repetition of test points. The studies yielded some intriguing results, but for future work it would be absolutely crucial to have a repetition of test points to verify the results presented in this thesis, and more importantly to have strong statistical support for any conclusions made from the data collected. Having strong statistics would also aid in creating an effective and accurate model of $SCCO_2$ extraction of lipids from an algal sample.

Another opportunity for future work would be to expand the scope of the analysis. The work presented in this thesis was focused on $SCCO_2$ extraction with the intent of the extract being used in biodiesel production. Although glycerides may account for the largest portion of lipids present in an algae cell [4], there are many other compounds that were not measured in this thesis. Glycerides can be transesterified into FAMEs, which is pure biodiesel, but what is not known is how other non-transesterifiable lipids affect the quality of the biodiesel. Some other lipids that could be included in an analysis would be carotenoids, xanthophylls, phenolics, terpenoids, sterols, or chlorophylls. Opening up the analysis of the extract to include more species of lipids would give a better understanding on the quality of the $SCCO_2$ extract for biodiesel, assuming the effect of these compounds on biodiesel is known. To analyze these other compounds it is likely that a combination of gas chromatography, high performance liquid chromatography, and mass spectrometry would be needed.

In addition to adding more analysis techniques to the extract, a rigorous analysis of the starting material would also be beneficial. Having complete knowledge of what is in the algal sample before extraction, and conducting the same analysis on the algal

sample after extraction would produce a very clear picture of the extraction process. Unfortunately, for this thesis there was not a known developed method for accurately analyzing the starting dried and ground algal sample. Knowing what is present before and after the extraction would allow for a detailed mass balance of the extraction as well as provide data for a model of the extraction.

The last aspect that could lead to a plethora of future work would be to use a cosolvent with the CO_2. A cosolvent could greatly change the nature of the extraction and the products that make up the extract. A cosolvent would add additional operational complication and complexity in understanding the results. Before work with a cosolvent is conducted, it is advised that a complete understanding of the neat CO_2 be achieved first. With the complete understanding of neat CO_2 it would then be much easier to interpret the results in order to understand how the cosolvent affects the extract from a supercritical extraction.

APPENDIX

The purpose of this appendix is to show the photos of the actual apparatus. Due to the complexity of the apparatus, sketches were used in the bulk text of this thesis.

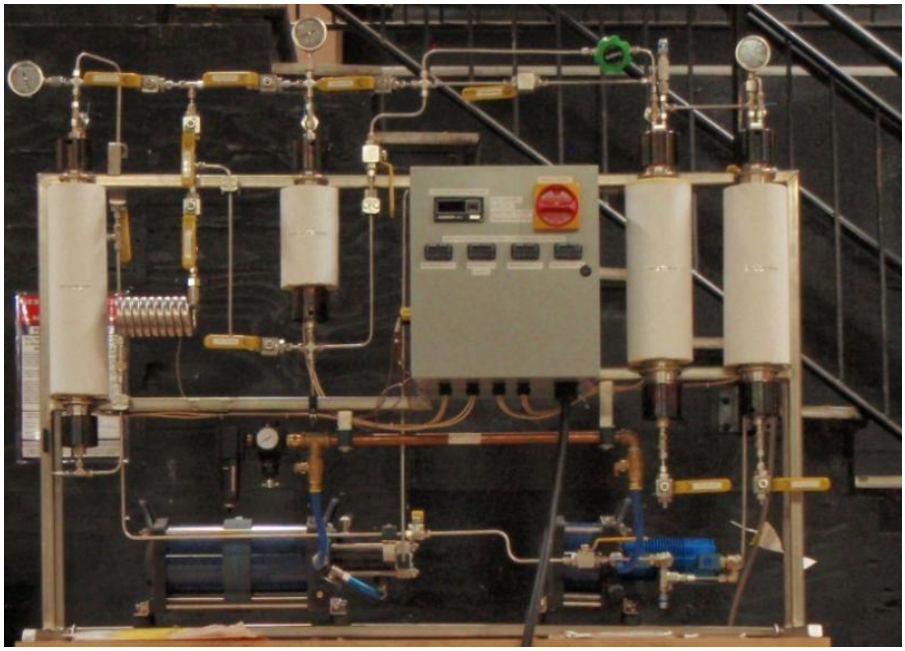

Figure 26. Photo of the apparatus for the Pilot Study. This is how the apparatus was originally received. All the main components are visible with the exception of the compressor, chiller, and CO_2 cylinder.

Figure 27. Photo of the air compressor that supplied the drive air for the air driven pumps.

Figure 28. Photo of the apparatus for the Equilibrium Study. This configuration had some tubing removed and the addition of the needle valve and polycarbonate box assembly.

Figure 29. Photo of the CO_2 collection bag for the Equilibrium Study inflated. This photo shows the toothpaste tube design of the bag.

Figure 30. Photo of the CO_2 collection bag for the Equilibrium Study fully rolled up.

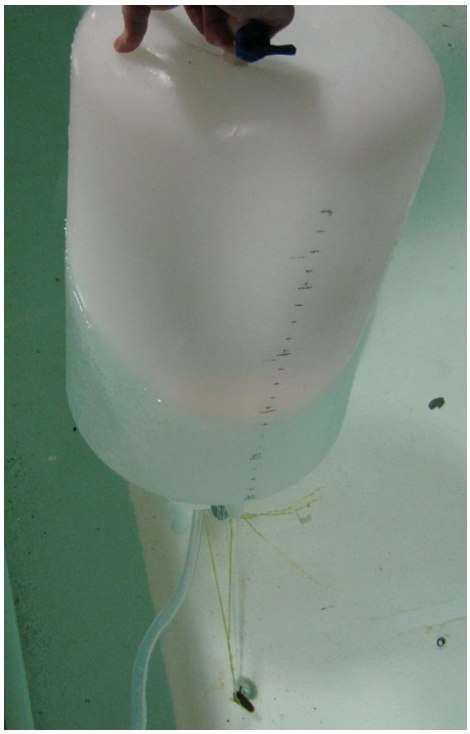

Figure 31. Photo of the CO_2 measurement device for the Equilibrium Study. The photo shows the graduations on the inverted carboy, and the tubing that would connect to the CO_2 collection bag.

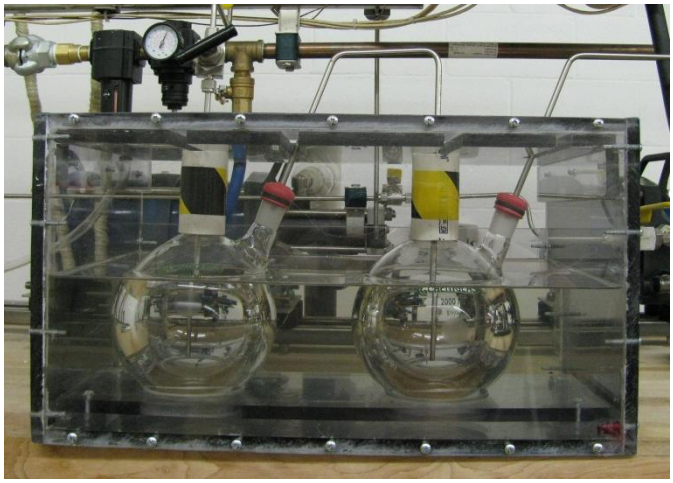

Figure 32. Photo of the polycarbonate box assembly for the Equilibrium Study. The photo shows the needle valve just above the top of the left collector. Short pieces of PVC were used to hold the flasks in place. The clear liquid in the box is water used to add a larger thermal mass.

Figure 33. Photo of the apparatus for the Flow Study. The reduction of tubing between the storage tank and back pressure regulator is very present in this photo. Also the polycarbonate box had to be reversed, so a plastic curtain was added for safety.

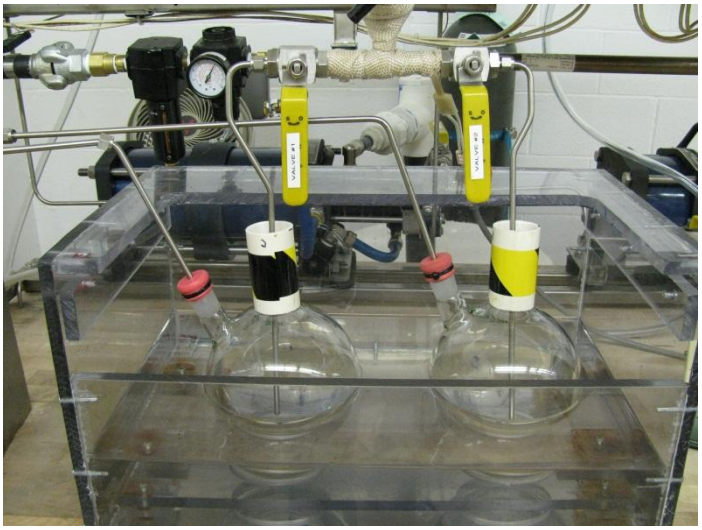

Figure 34. Photo of the polycarbonate box for the Flow Study with the top and curtain removed. At the top of the photo the bottom portion of the needle valve and the insulation is visible. This photo highlights the parallel configuration of the collectors.

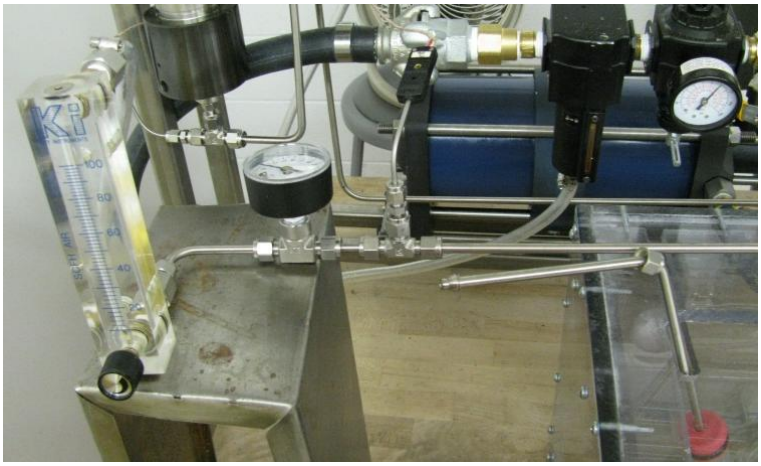

Figure 35. Photo of the CO_2 flow rate measurement equipment for the Flow Study. The tubing on the right side of the photo is the outlet from the collectors. To switch the measurement of collectors the tubing was manually connected/disconnected from the assembly.

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