One-Pot Algal Biodiesel Production in Supercritical Carbon Dioxide

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ABSTRACT

To advance the realization of algae as a feedstock for biodiesel, process technologies and closed-loop biomass use must be optimized. Life-cycle analysis (LCA) of the biodiesel production process highlights the potential significant impact of improvements in the extraction of algal lipids and conversion into biodiesel. Further, a single-step lipid extraction and transesterification process was shown to have the highest energy rewards. This work investigates the potential for using $scCO_2$ for the extraction and conversion process. $scCO_2$ is shown to be an effective and selective solvent for extracting triglycerides from algal biomass. This work also explores the fundamental science necessary to achieve a one-pot approach that both extracts and transesterifies lipid from algae using supercritical carbon dioxide/methanol ($scCO_2$/MeOH) and heterogeneous catalysts. A variety of basic and acidic heterogeneous catalysts have been surveyed for their effectiveness at transesterification of triglyceride (TG) to fatty acid methyl esters (FAME). Further the enhanced solubility of FAME over reaction intermediates, TG, and glycerol, is likely to provide a driving force for reaction. This research offers the foundations for a simple one-pot system wherein biodiesel can be directly, selectively, and sustainably produced from algae for further application in an algae biorefinery.

INTRODUCTION

In 2011 the expected global average consumption of crude oil and liquid fuels totaled 88.1 Mbbl/day with expected growth of ~1.5% in 2012 [1]. At the given oil usage rates, fossil oil peaking will likely begin within the next 40 years if it has not already [2, 3]. In order to meet the world's energy demands, alternative feedstocks must be developed into viable energy sources. In terms of transport fuels, algal biodiesel has the advantages of fast growth (with doubling times of 3.5 and 24 hours during the exponential growth [4]), low or marginal land use, and higher oil content per dry weight compared to plant derived biofuels. Further, they have the potential to utilize wastewater as a feedstock [5], to have geographic flexibility, and to establish a distributed, rather than centralized, system [6].

Oil content of some microalgae may be as high as 90% of dry biomass weight under extreme growth conditions [4], with concentrations between 20-50% being more common. These yields greatly exceed those of other oil crops such as soybean and oil palm with less than 5% lipid concentration [1]. The land area demanded by oil producing crops is at least an order of magnitude greater than microalgae on an energy per area basis [7]. Based on the estimate that algae contain 30% lipid biomass by weight and that lipid yields will be similar to those observed in photobioreactors, only 2.5% of current US cropland would need to be allocated to energy production [8]. Further, microalgae do not compete directly with food crops since they do not require fertile cropland nor are they a food resource. Water demand for algae is also shown to be up to 300 times less than that for soybean or 4.5 times less than that for oil palm per unit of biodiesel produced [9]. The potential use of wastewater for algal growth would further decrease the burden on freshwater resources [10].

Although algal biodiesel is preferential to crop-derived biofuels in terms of lipid yield, infrastructure, as well as water and land demand for production, the production costs and energy requirements need to be lowered to make algal biodiesel a truly competitive alternative fuel. While algal biofuels are not economically viable given the current refinery and subsidy systems, there is urgent need to design the next generation of renewable fuel sources with algae having tremendous potential as a sustainable and efficient alternative. As such, there is a significant incentive to improve and optimize the processes for algal biofuel production including lipid extraction and conversion.

Opportunities in the Algal Biodiesel Production Process.
The production of biodiesel from algae is a multi-step process that includes algal growth, harvesting, extraction, and conversion into fuel (Figure 1). In order for algal biofuels to become viable, technologies for each of these steps need to evaluated and optimized for efficiency and sustainability. Life-cycle analysis (LCA) of this process shows that there are significant energy gains to be had over the base case in terms of energy use particularly in the steps of harvesting and extraction/ conversion (Figure 1).

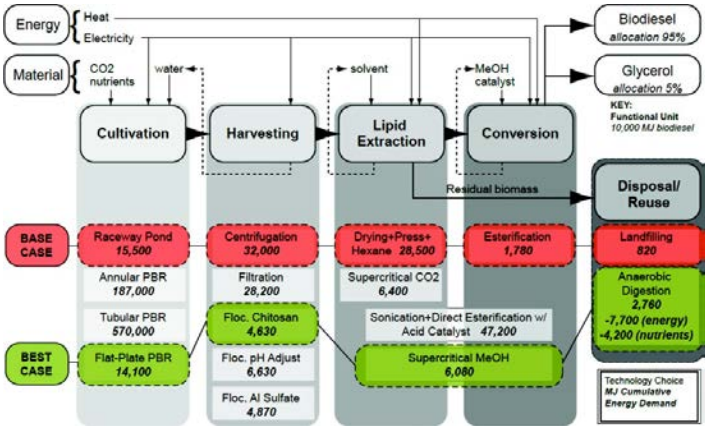

Figure 1: Life cycle analysis results of the energy requirements for algal biodiesel production [14]**.**

Current extraction methods, such as the Bligh and Dyer method [11], employ chloroform and/or hexane in a series of extraction and aqueous washing steps. The organic phase is then recovered, and upon evaporation of the chloroform, leaves a residue operationally defined as "lipid". This extraction process is non-selective as the "lipid" consists of several different components including hydrocarbons, polar lipids, pigments, in addition to the desired triglycerides (TG) [12]. While the Bligh and Dyer method is used for lipid analysis, it is non-selective, time consuming, wasteful, relies on toxic substances, and is not particularly efficient. In the base case scenario for commercial production of biodiesel (Figure 1), hexane extraction is considered the current industry standard due to the common use of hexane for use in other oil extractions [13]. This means of lipid extraction is also quite energy intensive as it requires a dry algae feedstock, and there is embedded energy associated with the production and distillation of hexane [14].

In the production of algal biodiesel, extraction is followed by a step-wise transesterification reaction producing three FAME molecules for every molecule of TG. Diglyceride (DG) and mono-glyceride (MG) are produced as intermediates, with glycerol as a by-product of the overall reaction (Figure 2). The first step of the reaction, TG to DG is generally considered the rate-limiting step in ambient conditions [15], but the reaction kinetics must be explored at supercritical conditions. Since complete transesterification of one TG molecule requires 3 molecules of alcohol, a minimal 3:1 molar ratio of methanol (or ethanol):substrate is required with kinetics being favored at ratios of at least 6:1 or higher [16].

In addition to an alcohol, the transesterification step utilizes an acidic, basic or enzymatic catalyst to carry out the reaction at temperatures below 100˚C. Acidic catalysts can be used in feedstocks that may be contaminated with free fatty acids or water, preventing saponification that would occur with basic catalysts. However, basic catalysts have faster reaction kinetics than acidic catalysts [16]. Lipases are also used for transesterification requiring a lower operating temperature, but are expensive compared to the alternatives [16].

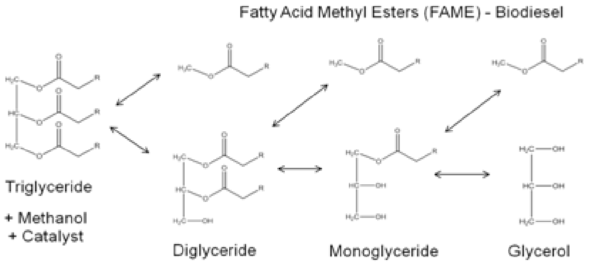

Figure 2: Transesterification reaction

Catalysts. In order to drive the reaction at minimal operating conditions, catalysts have been employed for transesterification. Alkaline, acidic, and enzymatic catalysts, homogeneous and heterogeneous, have all been explored for this application [17-22]. Homogeneous catalysts have been shown to work in all cases efficiently, but have significant barriers related to the contamination of the product and waste materials with these hazardous or expensive chemicals. Heterogeneous catalysts, however, can be easily recovered from the reaction and product mixture or embedded in a fixed bed for continuous operation and reuse [18]. Acidic and basic heterogeneous catalysts have been explored in a variety of solvents with varying degrees of success. Depending on the reaction conditions of temperature, alcohol: substrate ratio, catalyst loading, and time, as well as the catalyst properties of type, size, calcination/preparation time and temperature, up to a 96% conversion success of TG to FAME has been reported [18, 20]. The main downside to using heterogeneous catalysts is the decrease in efficiency in temperature requirements, yield, or kinetics. The application of supercritical fluids as the solvent for heterogeneously catalyzed transesterification can decrease this barrier as there is less resistance to mass transfer due to the decreased solvent viscosity [23].

Supercritical methanol (scMeOH) without a catalyst has also been used for triglyceride transesterification showing advantages in both system simplicity as well as reaction kinetics, but the higher operating temperature of at least 350˚C makes this option extremely energy intensive [24]. Despite the high temperature requirements, scMeOH may be the most energy efficient means to extract and convert lipid into biodiesel due to the decreased solvent and energy requirements for the single step process as well as the use of a wet algal feedstock [14]. While this technology has great potential, it still requires large amounts of energy to heat methanol past the supercritical point (239.1˚C) and is far from being commercially viable. Here we suggest using supercritical carbon dioxide with methanol as a co-solvent in order to produce a mixture that will be efficient at extraction, transesterification, and separation of products at much reduced operating temperatures, providing advantages seen through increased selectivity and solubility as well as decreased energy burden.

Supercritical carbon dioxide (scCO_2) has successfully been used commercially as a solvent for extraction, fractionation, and synthesis [25] with similar behavior to conventional solvents, hexane or ethyl acetate [26]. While it is not perfectly represented by either surrogate solvent, its properties allow it to efficiently and selectively extract non-polar compounds from complex matrices. There have been many studies [27-38] pertaining to lipid extraction by scCO_2 in the food industry, particularly for nutrient removal from plants and algae. Of particular interest are long chain hydrocarbon compounds known to be soluble in scCO_2 [30]. In algae, these products include valuable nutritive products such as carotenoids, chlorophylls, and ω3-fatty acids [28-32]. The effectiveness of lipid extraction from algae using scCO_2 is explored in this work in terms of lipid yield and product selectivity.

Further, scCO_2's selectivity offers a discrete advantage over conventional solvents during the conversion reaction in biodiesel. TG are moderately soluble while the FAME product is highly soluble (Figure 3) [39]. Compared to neat methanol where glycerol will not typically phase separate unless subjected to large drops in temperature or pressure [40, 41]), glycerol is almost insoluble in scCO_2 and will precipitate out of solution upon formation. These disparate solubilities will potentially be favorable for driving the reaction equilibrium forward due to a relative decrease in product concentration.

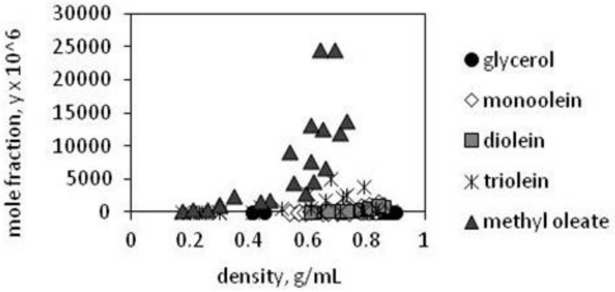

Figure 3: Solubility of oleate species in $scCO_2$. Data shown as a compilation [39] across density from a range of temperatures and pressures

Phase behavior. This research seeks to identify a reaction pathway (temperature, pressure, concentration) that allows for conversion of starting material to product with the desired phase behavior as seen for the pure methanol system at temperatures above 225˚C [40]. Previous work on the use of methanol as a solvent for TG transesterification employed supercritical temperatures and pressures, not so much because supercritical conditions were desired, but rather because the high temperatures (and consequently high pressures) were needed to create a single phase of methanol and non-polar TG. In our system we propose to employ a CO_2 and methanol mixture to allow for transesterification at more ambient temperatures. The fundamental understanding of the system's phase behavior and phase conditions for efficient reaction/extraction is explored.

MATERIALS AND METHODS

Algal growth. Algal cultures were grown in a small-scale reactor setup in media as recommended by [42]. 14 h/day of illumination will be provided with an artificial light source consisting of two fluorescent light bulbs per reactor vessel. The reactors were sparged with house air with an addition of 2% CO_2. The growth of each algae species was monitored by UV/Visible spectroscopy with cell density being correlated with absorbance at 610 nm as measured by direct cell count. 40 mL aliquots of algae were placed into 50mL Falcon tubes, centrifuged (12,000 rpm, 20 mins, 4˚C), the supernatant decanted, and the algae frozen at -20˚C until use. The wet algae mixture contains 4% solids with a total sample volume of 1 mL. The algae will then either be used wet or lyophilized to dryness and kept in a desiccator.

Extraction. Conventional solvent extraction was conducted with slight modifications to Bligh and Dyer[11] using 2:1 chloroform:methanol. Extraction using $scCO_2$ was performed using a supercritical fluid extractor (SFT-100, Supercritical Fluid Technologies). Samples were added to the extraction chamber, run continuously for one hour at specified pressure and temperature, and the flow through was collected at a rate of 4 SCFH of gaseous flow (~0.2 kg CO_2 per run).

Analysis. Using liquid chromatography-mass spectrometry (LC-MS) with atmospheric pressure chemical ionization (APCI), TG, DG, MG, glycerol, and FAME concentrations were measured. Mass spectrometry allows for the compounds to be identified by both retention time and molecular weight. Heptane with 0.1% acetic acid was used as the elution solvent to successfully separate FAME standards as well as several triolein, diolein, and monoolein [43]. The column used is a normal phase HILIC column (Waters) at a flow rate of 0.25 mL/min. Mass spectral settings were optimized for FAME with short, medium, and long chain lengths at capillary voltages of 75, and 110V and RF loadings of 80%, and 95% respectively. Standards are used to verify the presence and quantify each substrate.

Phase Behavior Determination. The phase behavior of the reaction mixtures was observed in a variable volume, high pressure view cell (D.B. Robinson and Associates; Edmonton, Alberta, Canada) as performed in previous works [44, 45]. Cloud point curves were measured isothermally. Methanol and substrate was added to the cell at a pre-determined ratio and the system sealed. Once the desired temperature is achieved, a measured quantity of CO_2 at is added at 1600 psi at room temperature (23˚C) isothermally and isobarically. Once the desired amount of CO_2 is added, the cell is pressurized to form a single phase. The cell is mixed at this pressure until equilibrium is achieved, and the cell is then depressurized until the cloud point is observed.

Reactor. A 50 mL reactor, equipped with a blade stirrer and aligned sapphire windows, was used for high pressure reactions. Typically, all reactants and catalyst were added into the reactor which is then sealed, purged with CO_2, and heated to the desired temperature. The reactor is then pressurized to the reaction conditions and stirred at 500 rpm. At the end of the reaction, the CO_2 is vented through a valve heated through a restrictor block and slowly bubbled through heptane for collection. The remaining contents of the reactor are sampled and all samples were analyzed on LC-MS.

RESULTS/ DISCUSSION

CO_2 as a solvent. Our results show that $scCO_2$ is efficient at extracting triglycerides with a FAME yield that is comparable to conventional organic solvents at select pressures and temperatures. The isobars seen in Figure 4 show that at 100˚C and 6000 psi, that the yield is comparable with that of conventional solvent extraction. Milder reaction conditions may be possible with the use of cell pretreatment or careful algae strain selection. [43]. In addition to being efficient at extracting the desired product of TG, $scCO_2$ is also selective against other undesirable compounds for fuel production such as pigments and phospholipids. Even at the most extreme conditions tested, $scCO_2$ extraction only had 15% of the pigment density (Figure 5) and no phospholipids in the fuel product as compared to extraction using conventional organic solvents. This selectivity is significant as pigments are undesirable in the fuel product [46] and phospholipids can poison the catalyst for transesterification [47]. An elemental analysis of the lipid extracts also shows an 18% reduction in the nitrogen content of the fuel product as compared to conventional organic solvents, important for the minimization of NO_x formation during combustion of the biodiesel (Table 1).

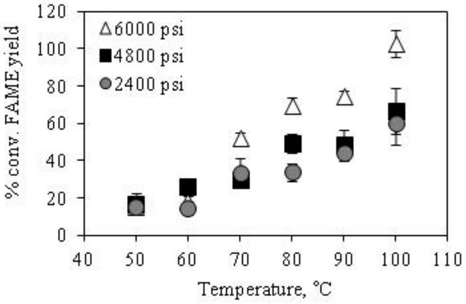

Figure 4: FAME yield from wet, frozen algae extracted by $scCO_2$ across temperature shown as a percentage of the conventional extraction yield for FAME on a per mass basis.

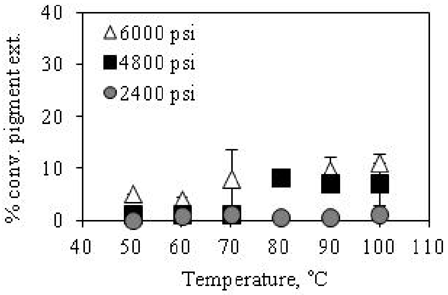

Figure 5: Isotherm data for pigment (pheophytin A) extracted with $scCO_2$ at varying temperature shown as a percentage of extract from conventional solvents.

Table 1: Elemental analysis of algal lipid extraction by conventional solvent and $scCO_2$ extraction at 4800 psi, 80˚C

	Conventional Extraction	Extracted with $scCO_2$
% C	70.44 ± 0.08	72.92 ± 0.41
% H	11.09 ± 0.10	11.77 ± 0.11
% N	0.58 ± 0.01	0.42 ± 0.04
Ratio H/C	0.157	0.161
Ratio N/C	0.0082	0.0058

Catalysts. A suite of catalysts were tested at 100˚C in methanol to assess their ability to transesterify triolein. Catalysts were chosen from literature [18, 20] representing effective catalysts for transesterification and inter-esterification and include basic, acidic, and neutral heterogeneous catalysts. The specific catalysts tested were Nafion, tungstosilicic acid hydrate, zeolites, CaO, K_2CO_3, hydrotalcite, and mesoporous silica (MCM-41). Yield is quantified based on mass balance and compared to positive controls (14% BF_3 in methanol and H_2SO_4).

Samples were treated similarly to standard transesterification using BF_3. Results of the kinetics and yields of several chosen catalysts show that all of the tested catalysts achieve ~100% FAME yield within 4 hours with maximum yield occurring for basic catalysts within the first hour. The four catalysts, zeolite, hydrotalcite, MCM-41, and Nafion were chosen as representative basic, neutral, and acidic catalysts to continue experimentation. Upon implementation in the supercritical reaction, several factors will need to be considered for improved reaction yield including catalyst particle size and mixing speed to decrease mass transfer requirements.

Phase Behavior. Experiments in a variable volume view cell indicated the phase behavior of the triglyceride, triolein, and its transesterification intermediates and products. These results show that while methanol increases the solubility of all of the substrates in CO_2 that the relative solubilites are unchanged meaning that the FAME, methyl oleate, remains significantly more soluble than the rest of the substrates and that glycerol is still mostly insoluble. Interestingly the solubility of monoolein and diolein seemed to have increased more than triolein, meaning that despite their limited solubility in neat CO_2, which could potentially cause reaction rate limitations, MG and DG solubility may not be an issue in the mixed system.

CONCLUSIONS

A More Optimal System: One-pot algal biodiesel production. Based on the current state of science and life cycle analyses, it is likely that a more optimal system for the production of algal biodiesel from a sustainability – economic, environmental, and social – perspective is a one-pot system using $scCO_2$, methanol, and heterogeneous catalysts. Realization of this system requires enhanced understanding of the fundamental science determining the phase behavior and reactivity of each of the system components. The idealized system would efficiently extract TG from the biomass and facilitate transport to the catalyst for transesterification with the solubilized alcohol (Figure 6). The glycerol by-product would be insoluble and the FAME could be easily recovered from the SCF stream by decreasing temperature and pressure. This work has provided some of the foundations for realizing this system in terms of catalyst selection and understanding the fundamental phase behaviors to determine substrate and methanol loadings as well as reaction conditions.

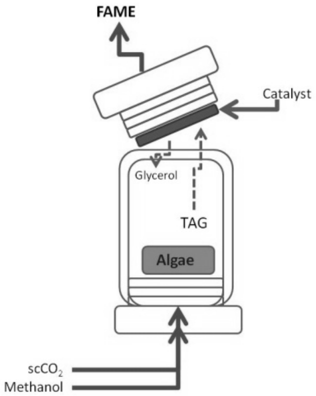

Figure 6: Depiction of possible reaction system configuration

In order to make algae a viable industrial feedstock, the other unused biomass portions must be used efficiently in pursuit of a biorefinery model [48]. The biorefinery concept, in which both fuels and multiple value-added co-products are pursued in parallel [49] has been proposed as a way to assuage many of these concerns [50]. This point of view is promoted by the United States Department of Energy (DOE) National Algal Biofuels Technology Roadmap, which cites the potential for valuable co-products as one of the key reasons for exploring algae as a source of biofuels [51]. In our view, the biorefinery approach is both compelling and necessary. In the same way that petroleum refineries maximize profits and material efficiency through value optimization of every chemical fraction, so too must algal biorefineries if they are to be a viable and competitive alternative. In addition to economic considerations, the biorefinery concept is in accordance with the principles of green engineering [52] and a sustainable energy infrastructure.

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