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Biodiesel Production from Wet Algal Biomass through in Situ Lipid Hydrolysis and Supercritical Transesterification

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In an effort to process wet algal biomass directly, eliminate organic solvent use during lipid extraction, and recover nutrients (e.g., N, P, and glycerol) for reuse, we developed a catalyst-free, two-step technique for algal biodiesel production. In the first step, wet algal biomass (ca. 80% moisture) reacts in subcritical water to hydrolyze intracellular lipids, conglomerate cells into an easily filterable solid that retains the lipids, and produce a sterile, nutrient-rich aqueous phase. In the second step, the wet fatty acid-rich solids undergo supercritical in situ transesterification (SC-IST/E) with ethanol to produce biodiesel in the form of fatty acid ethyl esters (FAEEs). *Chlorella vulgaris* grown sequentially under photo- and heterotrophic conditions served as the lipid-rich feedstock (53.3% lipids as FAEE). The feedstock and process solids were characterized for lipid components using highly automated microscale extraction and derivatization procedures and high-temperature gas chromatography. Hydrolysis was examined at 250 °C for 15 to 60 min; solids recovered by filtering contained 77−90% of the lipid originally present in the algal biomass, mainly in the form of fatty acids. The effects of reaction time (60 or 120 min), temperature (275 or 325 °C), and ethanol loading (approximately 2−8 w/w EtOH/solids) were examined on the yield and composition of biodiesel produced from the SC-IST/E of the hydrolysis solids. Longer time, higher temperature, and greater ethanol loading tended to increase crude biodiesel and FAEE yields, which ranged from about 56−100% and 34−66%, respectively, on the basis of lipid in the hydrolysis solids. Isomerization and decomposition of unsaturated FAEEs was quantified, and its effect on fuel yield is discussed.

Introduction

Worldwide energy demand, particularly for liquid transportation fuels, continues to rise as populations grow and become more affluent. Concurrently, concern over climate change, declining petroleum reserves, and national security has encouraged the use of biodiesel, a mixture of fatty acid alkyl esters commonly derived from the transesterification of vegetable oils. Biodiesel is an attractive replacement for petroleum diesel because it is domestically available, biodegradable, compatible with existing diesel engines, and reduces tailpipe emissions of most criteria air pollutants.[1,2] Recent interest in using oleaginous microalgae as a nonedible biodiesel feedstock has grown considerably, largely on the promise of high oil yields (5,000 to 100,000 L/ha-y), the opportunity to capture waste CO_2, and the ability to cultivate algae on abandoned or unproductive land using brackish, salt, or wastewaters instead of freshwater.

Some microalgae respond to certain chemical and physical stimuli through the accumulation of intracellular triglycerides (TGs), the most common parent material for biodiesel.[3] Unlike terrestrial oilseeds (e.g., soy), which can be easily harvested, dehulled, flaked, and dried to ∼10% moisture prior to extraction with *n*-hexane, microalgae grow in dilute aqueous suspensions that complicate lipid recovery. Microalgae grown outdoors in open ponds typically have cell density and productivity ranging from 0.5 to 2 g dry biomass/L and 10−40 g/m^2-d, respectively.[4] Though higher biomass densities (5−200 g/L) can be achieved in thin-plate photobioreactors[5,6] and fermentors,[7,8] dewatering and drying remain energy- and cost-intensive processes.[9,10] A recent life-cycle assessment (LCA) of algal biodiesel production from *Chlorella vulgaris* indicated that drying and hexane extraction accounted for up to 90% of the total process energy.[11] These data indicate that drying algal biomass and treating it as a substitute for terrestrial oilseeds in traditional solvent extraction and subsequent transesterification processes is not likely to be a net energy positive route toward sustainable biodiesel production.

A biodiesel production process that obviates biomass drying and organic solvent use for oil extraction could lead to significant energy and cost savings. Previous attempts to eliminate even one of these steps have met with only limited

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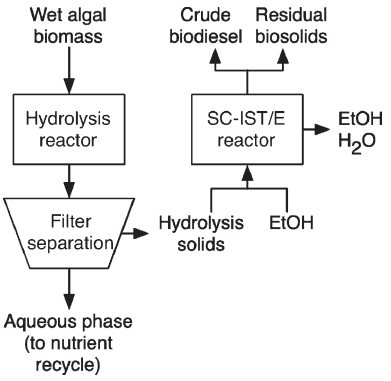

Figure 1. Process flow diagram for biodiesel production through intracellular lipid hydrolysis and supercritical in situ transesterification (SC-IST/E) using ethanol (EtOH).

success. For example, efforts to combine extraction with acid-catalyzed transesterification in one step have been successful with dry algal biomass, but the reaction is severely inhibited by water.[12,13] Herein we propose a two-step, catalyst-free biodiesel production process involving intracellular lipid hydrolysis coupled with supercritical in situ transesterification (SC-IST/E). Figure 1 provides a process schematic. In the first step, wet algal biomass (ca. 80% moisture) reacts at subcritical water conditions to hydrolyze intracellular lipids, conglomerate cells into an easily filterable solid that retains the lipids, and produce a sterile, potentially nutrient-rich aqueous phase. In the second step, the wet fatty acid (FA)-rich solids are subjected to SC-IST/E with ethanol to produce biodiesel in the form of fatty acid ethyl esters (FAEEs). This process eliminates both biomass drying and TG extraction (e.g., with an organic solvent such as *n*-hexane). Several factors motivate this approach: (1) Oil hydrolysis is a well-known commercial process and can be carried out under mild subcritical water conditions;[14] (2) supercritical esterification can be performed at lower temperatures, in less time, and can achieve higher conversions compared to supercritical transesterification;[15,16] (3) amassing cells into a filterable solid accomplishes additional dewatering prior to transesterification with minimal costs; (4) retention of FAs and remaining lipids in a solid matrix obviates difficulties with lipid recovery from aqueous systems that can require energy-intensive (e.g., centrifugation and evaporation),[16] hazardous (e.g., solvent recovery),[14,17] and expensive (e.g., enzyme)[18] treatments for separation; (5) nutrients (e.g., N and P) and glycerol from processed biomass can be captured and reused in a sterile aqueous phase free of catalyst; and (6) a well-engineered process to produce biodiesel through supercritical alcohol transesterification may reduce costs and energy expenditure relative to those of conventional catalytic methods.[19,20]

The hydrothermal conversion of various biomacromolecules has been well studied,[21] but to our knowledge, the in situ conversion of cellular lipids to FA by subcritical water hydrolysis has never been investigated in oleaginous microalgae. Given that algae accumulate TG-rich, cytoplasmic lipid bodies and contain phospholipid membranes, we hypothesize that it is possible to convert these lipids to FA in subcritical water. In the second step, FAEE are generated through the SC-IST/E of FA and the remaining glycerides within the wet hydrolysis solids with ethanol. The literature records only one previous attempt to process lipid-containing biomass directly in supercritical alcohols.[22] Ethanol is attractive because it can be derived from renewable sources, is less toxic than methanol, and is generally recognized as safe, an important consideration for the use of residual aqueous phase or solids. Furthermore, FAEEs typically demonstrate slightly higher cetane numbers, improved low temperature operability, and greater oxidative stability when compared to fatty acid methyl esters (FAMEs).[23−25]

To our knowledge, all attempts to synthesize biodiesel involving the hydrolysis of oil to FA and subsequent esterification with supercritical alcohols have employed anhydrous oil, methanol, and complex phase separation.[16,26] In this article, we demonstrate for the first time that it is possible to carry out in situ hydrolysis of cellular lipids in wet algal biomass, retain those lipids within a filterable solid, and then produce biodiesel through in situ transesterification using supercritical ethanol (SC-EtOH).

Experimental Section

Feedstock Production. Lipid-rich *Chlorella vulgaris* was grown as a biodiesel feedstock using sequential photo- and heterotrophic growth stages. *C. vulgaris* (University of Texas Culture Collection of Algae #259) was first grown phototrophically in a series of six bubble column reactors (3.8 cm diameter × 130 cm length each) continuously fed (approximately 60 mL/h) with a modified Bold's Basal Medium (concentration, mg L^{-1}): $NaNO_3$ (750), $CaCl_2 \cdot 2H_2O$ (25), $MgSO_4 \cdot 7H_2O$ (75), K_2HPO_4 (75), KH_2PO_4 (175), NaCl (25), $Na_2EDTA \cdot 2H_2O$ (4.5), H_3BO_3 (2.86), $FeCl_3 \cdot 6H_2O$ (0.58), $MnCl_2 \cdot 4H_2O$ (0.25), $CuSO_4 \cdot 5H_2O$ (0.079), $ZnCl_2$ (0.030), $CoCl_2 \cdot 6H_2O$ (0.012), $Na_2MoO_4 \cdot 2H_2O$ (0.024), vitamin B_{12} (0.131), biotin (0.024), and thiamin (0.534). The synthetic medium was prepared with purified deionized water and adjusted to pH 7.2 with 1 N NaOH. A humidified 2−3% CO_2−air mixture was delivered to each column (0.3 L/L-min) via a linear air pump (LT19, Whitewater) and a regulated CO_2 tank. Light was supplied continuously with cool-white fluorescent bulbs (SP-50 ECO, 32 W, GE), for a light output at the tube surface of approximately 250 μmol/m^2-s. A PAR detector (MQ-303, Apogee Instruments) was used to measure irradiance. The apparatus was maintained at room temperature (23−25 °C). The mean hydraulic residence time in the reactor was 7 days. Reactor effluent (~3.5 L) was collected in a 10 L HDPE carboy for 2 days, then transferred to a 4 L flask. Glucose (20 g/L) was added to the flask, and the cells were then cultivated in the dark with aeration (1 L/L-min) and stirring for 7 days.

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Biomass dry weight was measured gravimetrically following centrifugation of the culture media (2000 RCF, 15 min) and drying of the wet algal pellet (65 °C for 24 h). The supernatant was retained to measure pH and NO_3^- concentration according to standard methods.[27] Heterotrophic cells collected by centrifugation were dried, ground briefly (< 15 s) with a mortar and pestle to obtain a homogeneous powder, and stored at 4 °C prior to use.

In Situ Lipid Hydrolysis and Supercritical in Situ Transesterification (SC-IST/E). All high temperature reactions with wet algal biomass were carried out in 316 stainless steel (SS) reactors fashioned from Swagelok parts (2 caps and 1 port connector). Once loaded, reactors were immersed in a preheated, isothermal fluidized sand bath for a desired time and then promptly removed and cooled in water. Hydrolysis reactions at each condition were carried out simultaneously in two reactors. The larger reactor (10 mL) was loaded with dry algae (1 g) and water (4 g) and reacted for 15, 30, 45, and 60 min at 250 °C. Some additional reactions were carried out at 225 and 300 °C. Drying and then rehydrating the biomass allowed for improved sample preservation during storage and the precise control of solids loading in hydrolysis reactions. Drying and cold storage of algal biomass was used in this work solely for convenience in the laboratory; a commercial scale process would use freshly harvested wet algal paste. No difference in the solids yield, elemental composition, or moisture content of hydrolysis solids was found when reactions were completed with freshly harvested biomass.

Upon cooling, the aqueous phase and solids were separated by filtering under light vacuum (predried, preweighed 934-AH filter paper, Whatman). No additional water was used to rinse the reactor housing. For some reactions, the aqueous phase was hexane-extracted to detect lipid components. The wet hydrolysis solids were stored at 4 °C prior to use; a portion of these solids was dried to determine its moisture content. The yield of hydrolysis solids was determined from a parallel reaction in which dry algae (0.5 g) and water (2.0 g) were loaded into smaller reactors (4 mL). Upon cooling, 15 mL of H_2O was used to ensure complete solids recovery from the reactor. Filter-separated hydrolysis solids were then dried (65 °C, 24 h) and massed.

SC-IST/E was carried out in 1.6 mL of SS reactors with wet hydrolysis solids (60 mg) derived from the hydrolysis reaction at 250 °C for 45 min. An exploratory 2^3 factorial experiment was carried out to determine the effects of reaction temperature (275 and 325 °C), reaction time (60 and 120 min), and ethanol loading (~2 and 8 w/w EtOH/solids) on the yield and composition of crude biodiesel. Following the reaction, reactor contents were filtered as described above; ethanol (95%; 15 mL) was used to wash the reactor. Hexane (1 mL) was added to the empty reactor to ensure complete ester recovery without exposing solids to this solvent. The ethanol-biodiesel mixture was collected in a round-bottom flask, and ethanol was evaporated under vacuum at 70 °C. The crude biodiesel was resuspended in hexane (5 mL), combined with the 1 mL hexane reactor wash, centrifuged to remove any remaining fine particles, and transferred to a sample vial. The hexane−biodiesel mixture was analyzed for FAEEs directly by gas chromatography with flame ionization detection (GC-FID), and the mass of crude biodiesel was determined gravimetrically, as described below. The crude biodiesel (5−10 mg) was then analyzed for FAs, monoglycerides (MGs), diglycerides (DGs), TGs, and glycerol following derivatization with *N*-trimethylsilyl-*N*-methyl trifluoroacetamide (MSTFA, Sigma), as described below. Each reaction condition was analyzed in duplicate. Response variables were analyzed using analysis of covariance with $p < 0.05$ (Minitab 15.1.30.0).

Lipid Analysis via Solvent Extraction. Lipids within algal biomass and hydrolysis solids were extracted and analyzed to determine their composition (relative amounts of FA, TG, DG, and MG). Dry solids (~30 mg) were loaded into glass tubes (16 mm × 100 mm) with Teflon-lined screw caps and extracted at 60 °C for 4 h with 6 mL of *n*-hexane or a mixture of *n*-hexane/isopropanol (HIP, 3:2 v/v). *n*-Hexane was chosen on the basis of its commercial relevance in oilseed extraction and previous use to extract heterotrophic algal lipids,[7,28−31] whereas HIP was employed as a less toxic alternative to chloroform/methanol mixtures.[32] Upon cooling, the tubes were centrifuged (2000 RCF, 15 min), and 2 mL of the upper solvent layer (i.e., crude lipid extract, CLE) was transferred to a 2 mL GC-vial and stored at 4 °C prior to analysis.

The CLE (50 to 500 μL) was transferred to two preweighed GC vials using the Agilent 7693A Automatic Liquid Sampler. The solvent was evaporated under N_2 (Visiprep Solid Phase Extraction Vacuum Manifold, Supelco), and the mass of the CLE was determined gravimetrically (XS205DU, Mettler Toledo, readability = 0.01 mg). Prior to analysis by high-temperature (HT) GC/FID, the CLE was derivatized with MSTFA to improve the volatility of lipid components containing free hydroxyl groups (e.g., FA, MG, and DG). A modified microscale EN14105 procedure was adopted to automate standard and sample preparation, include FA quantification, increase analysis throughput, and reduce experimenter exposure to hazardous solvents.[33,34] Three internal standards (1,2,4-butanetriol, dodecanoic acid, and tricaprin) in pyridine (10 μL each; 6,000−8,000 ppm) and MSTFA (20 μL) were added to a vial, and the vial was stirred for 1 min and then allowed to react for 30 min at room temperature. The sample was diluted in *n*-heptane (700 μL), stirred again, and injected in an Agilent 7890 GC-FID with an ASTM6584 column (15 m × 320 mm × 0.25 μm, Agilent J&W). Injection (1.0 μL) was made to a cool-on-column inlet in oven-track mode with an initial oven temperature of 50 °C. After a 1 min hold, the temperature was ramped to 180 at 15 °C/min, 230 at 7 °C/min, and 380 at 30 °C/min. Helium was the carrier gas at a constant flow rate of 3.0 mL/min. FID detector temperature was 380 °C, and N_2 served as the makeup gas (30 mL/min). Peaks corresponding to FA, MG, DG, and TG were identified by their retention time and quantified on the basis of internal standard calibrations of oleic acid, monoolein, diolein, and triolein, respectively.

Lipid Analysis via Acid-Catalyzed in Situ Transesterification. Lipids from all classes in algal biomass, hydrolysis solids, and residual solids from SC-IST/E reactions were simultaneously extracted and catalytically transesterified to determine the total lipid content. Since acid-catalyzed in situ transesterification has repeatedly been shown to recover more FAs than traditional two-step extraction and transesterification procedures,[35−38] this procedure provided the theoretical maximum ester yield possible from process solids. This method has been applied successfully to dry algae on both an analytical[37−40] and larger scale.[12,13]

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Dried algae (30 mg) or hydrolysis solids (15 mg) were weighed into glass tubes (16 mm × 100 mm) with Teflon-lined screw caps and reacted with 2 mL of freshly prepared ethanol (99%) containing 5% acetyl chloride at 100 °C for 90 min with vigorous stirring. Water (1 mL) was added to stop the reaction, and FAEEs were extracted into 6 mL *n*-hexane. The tubes were vortexed for 1 min and centrifuged (2000 RCF, 10 min). Approximately 2 mL of the upper hexane−FAEE mixture were transferred to a GC vial. FAEEs were identified and quantified by GC-FID with a modified version of EN14103,[41] and the mass of biodiesel was determined gravimetrically in a manner similar to the CLE. A new vial containing the sample (195 μL) and internal standard (5 μL, tricosanoic methyl ester, C23:0 FAME, Supelco) was prepared by the 7963A Automated Liquid Sampler and injected (1 μL; 15:1 split ratio; 260 °C inlet temperature) onto an HP-InnoWax column (30 m × 0.32 mm × 0.25 μm, J&W 1909BD-113) with an initial oven temperature of 170 °C. After a 3 min hold, the temperature was ramped at 3 °C/min to 250 °C. Helium was the carrier gas at a constant flow rate of 1.0 mL/min. FID detector temperature was 260 °C, and N_2 served as the makeup gas (30 mL/min). FAEE were identified on the basis of retention time using a C4−C24 FAEE mix (Supelco); total FAEE calculations reflect all integrated area between FAEE C14:0 and C24:0, as designated by EN14103. Vials containing pure *n*-hexane served as negative controls (gravimetric yield on blanks was never greater than 0.1 mg).

Results and Discussion

Feedstock Production and Characterization. In an effort to produce lipid-rich algal biomass, *C. vulgaris* was grown phototrophically in a series of continuously fed bubble column reactors and then heterotrophically in a single stirred tank. Sequential phototrophic and heterotrophic growth phases were employed to increase biomass density, lipid productivity, and carbon substrate utilization efficiency, as has recently been demonstrated in cultures of *C. protothecoides*.[42] Lipid accumulation was promoted through N stress; nitrate in the media was entirely consumed by the fourth bubble column reactor.

Green cells collected at approximately 2 g/L were then grown on glucose in the dark for 7 days, during which time they yellowed, and biomass density increased to about 4 g/L. Under photosynthetic conditions, a reduction in chlorophyll in the absence of external N has been hypothesized to correspond to intracellular scavenging of this N-rich compound,[43,44] while chlorophyll breakdown and chloroplast degeneration during heterotrophy have been linked to lipogenesis in *C. protothecoides*.[42]

Dry algal biomass after both the phototrophic and heterotrophic growth stages was thoroughly characterized. The biomass total lipid content, measured as FAEE by GC-FID, increased only slightly from 50.3 to 53.3% during heterotrophic cultivation. This total lipid content was higher than the previously reported range (21−36%) for *C. vulgaris* grown in media containing 1−2% of acetate, glycerol, or glucose[45] but similar to gravimetric lipid determinations

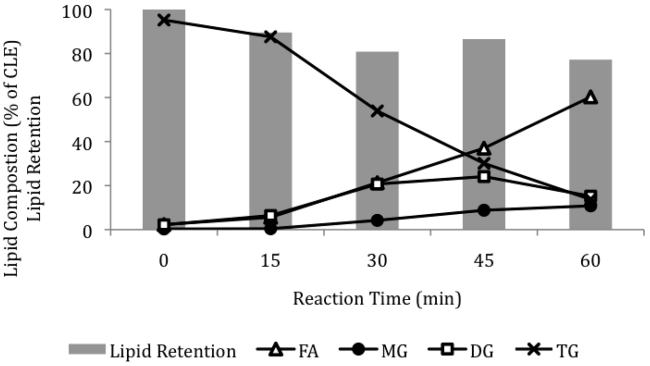

Figure 2. Temporal variation of lipid composition and lipid retention in solids recovered from hydrolysis at 250 °C. Lipid composition is the proportion of fatty acids (FAs), monoglycerides (MGs), diglycerides (DGs), and triglycerides (TGs) in the crude lipid extract (CLE) obtained by hexane/isopropanol extraction. Lipid retention was calculated from the dry weight yield of hydrolysis solids and the lipid content of both algal biomass and hydrolysis solids as determined by their fatty acid ethyl ester composition from GC-FID analysis of acid catalyzed in situ transesterification. Time zero represents the heterotrophic algal biomass before hydrolysis.

reported for N-starved *C. vulgaris* grown phototrophically with CO_2 supplementation (40−50%)[46,47] and in a marine medium supplemented with iron (56%).[48] The lipid content achieved compares favorably to that measured in high-cell-density cultures of heterotrophic *C. protothecoides* (40 to 50%), a related alga more routinely researched for biodiesel feedstock production.[7,49] The proportion of TGs in lipids recovered from biomass harvested after heterotrophic cultivation was higher than that of cells grown in light only (cf. 95.2 ± 0.1 and 92.7 ± 0.3%), while the proportion of FA was slightly lower (cf. 2.3 ± 0.07 and 3.6 ± 0.25%). As indicated by the results shown for time zero in Figure 2, the feedstock employed in hydrolysis reactions contained 95−96% TG, 2−3% DG, <1% MG, and 1−2% FAs. Hexane and HIP were used to extract neutral and total lipids, respectively. As expected, HIP resulted in greater CLE yields (wt% of solids) from the heterotrophic biomass relative to hexane extraction (cf. 45.6 ± 3.4 and 37.0 ± 2.9), but surprisingly no such trend was observed in the extraction of photosynthetic biomass (cf. 39.1 ± 5.4 and 39.6 ± 5.6). Table 1 shows that, as expected, the algal biomass FAEE yields of ∼53% exceeded the CLE yield from solvent extraction, even when accounting for the ca. 5.3% mass increase of ethyl esters relative to that of their parent TGs.[50] In both procedures, the average percent difference between gravimetric and GC determinations was less than 5% and within the experimental standard deviations, suggesting good instrument performance and minimal inclusion of nondetected impurities.

The FA profile of biodiesel feedstocks is an important determinant of fuel quality.[23,51] Table 1 shows that the major FAs of harvested cells were 46% oleic acid (C18:1), 18% palmitic acid (C16:0), 13% linolenic acid (C18:3), and 9%

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Table 1. Characterization of Feedstock and Hydrolysis Solids[a]

		hydrolysis solids reaction time (min)			
	feedstock	15	30	45	60
Ester Yield (wt%)					
gravimetric	52.9 ± 1.9	78.7 ± 4.1	70.8 ± 8.0	73.5 ± 2.8	73.0 ± 3.1
GC-FID	53.3 ± 1.3	77.1 ± 2.1	74.8 ± 1.1	73.1 ± 1.0	69.4 ± 0.4
Fatty Acid (% of Esters)[b]					
C14:0	0.4	0.5	0.2	bdl	bdl
C16:0	17.8	18.2	17.8	17.8	18.7
C18:0	2.1	2.1	2.3	2.0	2.2
C18:1	45.5	46.8	47.0	45.5	47.7
C18:2	9.0	9.2	9.1	8.7	9.0
C18:3	12.7	12.8	12.6	12.8	12.7
Crude Lipid Yield (wt%)[c]					
gravimetric	45.6 ± 3.4	84.3 ± 1.7	85.4 ± 1.5	82.8 ± 0.4	82.7 ± 3.2
GC-FID	47.1 ± 2.3	82.1 ± 4.0	82.0 ± 1.8	77.4[d]	77.4 ± 3.0
Elemental Analysis (wt %)[e]					
C	58.06	73.9	73.29	72.98	73.88
H	9.05	10.71	10.27	10.28	10.63
N	1.16	0.76	0.96	0.95	0.92
O	28.61				
H/C atomic ratio	1.87	1.74	1.68	1.69	1.73
N/C atomic ratio	0.017	0.009	0.011	0.011	0.011
% C retained	100	78.8	72.7	79.3	75.5
% N retained	100	40.6	47.7	51.7	47.0

[a] Standard deviation is given for the mean of duplicate reactions/extractions; ester percentages all had a standard deviation less than 0.6%. [b] Other fatty acids detected, present at less than 5% of total fatty acids, included: C12:0, C16:1, C17:0, C20:0, C22:0, and C24:0. The percentages of C18:1, C18:2, and C18:3 include all isomers. [c] Crude lipid extracted with hexane-isopropanol (3:2). [d] Replicate data was lost. [e] All elemental analysis data represent single analyses performed by Atlantic Microlab, Inc. (Norcross, GA). bdl = below detection limit.

linoleic acid (C18:2). This FA distribution is similar to that reported for N-starved *C. vulgaris,*[44] but with more oleic acid and less palmitic acid than some recent reports.[52,53] Incubation with glucose in the dark decreased the percentage of C18:3 (cf. 18.3 and 12.7%) and increased the percentage of C18:1 (cf. 36.6 and 45.5%) FAs, resulting in a FA profile more likely to yield biodiesel with improved cold-flow properties and oxidative stability.[51] This finding corroborates evidence that FA biosynthesis in heterotrophic *C. vulgaris* stops at oleic acid[54] and suggests that combined photo- and heterotrophic cultivation can be used to produce algal biomass with a suitable FA profile for biodiesel.

Elemental analysis showed that the N content of harvested cells was very low (1%) compared to reported values for the N content of microalgae (e.g., 5−9%), most likely reflecting the decrease in protein and increase in lipids typically observed during N limitation and heterotrophy.[30,47] In addition, since the N in the media was consumed by the fourth lit reactor, and biomass density increased sharply during heterotrophic growth, there must have been a concomitant decrease in the biomass N-to-lipid ratio. Such an effect is desirable because it indicates an efficient use of N to produce lipids and reduces the potential for N-containing fuel impurities (e.g., chlorophylls) that could lead to increased NO_x emissions during combustion. The problem of NO_x emissions is a critical factor in why we initially chose to produce liquid fuels from carbonized algal biomass, as opposed to investigating its direct combustion as was recently reported.[55] The elemental analysis results in Table 1 lead to an approximate molecular formula for ash-free dry algae of $C_{5.9}H_{11.0}O_{2.2}N_{0.1}$ and a theoretical chemical oxygen demand (COD) of 2.77 g COD/g volatile solids. Using the Dulong formula and neglecting the contribution of sulfur,[56] we estimate the heat of combustion for the dry algal solids to be 27.3 MJ/kg.

In Situ Lipid Hydrolysis. Wet algal biomass was hydrothermally processed to hydrolyze intracellular lipids, conglomerate biomass into a filterable solid, and generate a sterile, potentially nutrient-rich aqueous phase. The lower temperature limit for hydrolysis was determined in exploratory experiments that indicated the formation of a black, charcoal-like filterable solid above 225 °C but not at lower temperatures. We suspect that conglomeration is due to dehydration, condensation, polymerization, and aromatization reactions of polysaccharides in the cell wall and extracellular matrix. The upper temperature limit for hydrolysis was selected to limit excessive FA transisomerization and potential degradation of unsaturated FA that could impact fuel quality.[14,17,57] On the basis of these reports, 250 °C was chosen for this initial investigation of how intracellular lipids changed during hydrolysis.

The literature indicates that subcritical water hydrolysis of vegetable oils is predominately a homogeneous reaction in the oil phase,[58] consisting of three reversible, stepwise reactions that convert TG into DG, MG, and finally glycerol. The rate of hydrolysis is typically slow at first and then increases as FAs are formed. This increase has been attributed to the increased solubility of water in FA compared to TG[59] and the autocatalytic effect of FA.[60] The algal lipids we processed contained about 1−2% FA initially; therefore, these could lead to more rapid hydrolysis compared to a

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system containing TG alone. Our exploratory experiments at 225 °C indicated that very limited hydrolysis occurred compared to the data we report here for hydrolysis at 250 °C. These results are in agreement with findings that the rate of TG hydrolysis increases with temperature as water's ion product increases, dielectric constant decreases, and it becomes more soluble in the oil phase.[16,61] Of course, kinetic effects are also important. The equilibrium conversion from hydrolysis increases with the water-to-oil ratio in the feed. Higher water-to-oil ratios, however, reduce TG and FA concentrations, which can decrease the initial reaction rate[62] and increase operating and capital costs because a larger volume of material must be processed. The wet algal biomass we reacted contained about 20% solids and 50% lipids on a dry weight basis. If we model the feedstock lipids as triolein and assume a pseudohomogeneous reaction mixture, the water-to-oil ratio in our experiments was 6.8 (v/v), 8.0 (w/w), and 393 (molar). The large molar excess of water indicates that a very high equilibrium conversion should be attainable from hydrolysis under these conditions. The reaction mixture is not completely homogeneous, however, and equilibrium conversion will no doubt be affected by the relative proximity of intracellular lipids and water. Future work exploring how the concentration of water and glycerol (a hydrolysis product) vary within the lipid body and more widely across the cell could provide important information about how compositional heterogeneity and diffusion-limited transport affects the reaction.

Hydrolysis solids contained approximately 77−85% and 70−79% total lipids measured as the HIP CLE and FAEE, respectively (Table 1). Unexpectedly, FAEE yields were lower than the yield of the HIP CLE. Although unlikely, it is possible that the acid-catalyzed in situ transesterification did not go to completion or that nonlipid constituents liberated during hydrolysis were extracted into the CLE and perhaps coeluted with detected lipid components. As shown in Figure 2, TG content decreased, and FA content increased with time when wet algal biomass was reacted at 250 °C. The content of MG also increased with time, while DG concentration increased up to 45 min, reached a maximum, and then declined. The trends apparent in Figure 2 are what one would expect for a series reaction such as TG hydrolysis. As also shown in Figure 2, the total lipid retention (wt % of lipids in the dry algal feedstock loaded into the reactor that remained in the solids recovered after hydrolysis) varied from 77 to 90%, with the highest retention occurring at the shortest reaction time. Run-to-run variability in the lipid retention data, mainly due to solids yield, requires further study. The net effect of this hydrothermal treatment for 60 min was to hydrolyze approximately 86% of the TGs in the feedstock and produce easy-to-recover solids containing ∼80% of the total lipids, highly enriched in FA.

Table 1 displays the FA profiles for the lipids in the hydrolysis solids recovered at each different reaction time. There was little variation in the distribution of the major FAEE with reaction time or in comparison to the original algal feedstock. This result indicates that there is no selective retention or rejection of certain FAs during the hydrothermal treatment. However, we did see evidence for isomerization and minor decomposition of linolenic acid (C18:3). About 10.4, 13.0, and 16.6% of the total C18:3 FAEE detected in reactions lasting 30, 45, and 60 min, respectively, were transisomerized. At 60 min, the percent difference between the yield of all C18:3 isomers and the average yield of saturated FAEE was 3.5%, suggesting some decomposition may have occurred at these longer times.

Table 1 also provides the elemental analysis of the hydrolysis solids. The C, H, and N content showed very little variation with time. The hydrolysis solids were richer in C and H but had reduced amounts of N compared with those in the dry algal biomass feedstock. Between 73 and 79% of the C and 41−52% of the N in the algal biomass feedstock were retained in the hydrolysis solids. The yield of hydrolysis solids (% of mass in dry algal feedstock loaded into the reactor that remained in the solids recovered after hydrolysis) was always about 60% regardless of the reaction time. This solids yield is in the range desired as it is high enough to include essentially all of the lipids but low enough that most nonlipid components became dissolved in the aqueous phase. These data compare favorably to the solids yields and C retention from hydrothermal reactions carried out on *Dunaliella salina* (5−25% solids) at 190−210 °C, which varied from 25 to 45% and 38 to 62%, respectively.[55] A similar enrichment in carbon has been observed for the hydrothermal carbonization of cellulose at 200−250 °C for 2 to 4 h; C increased from 44.4% in the parent material to 70.7−72.7% in the hydrochar with solids yields and C retention in the range of 30−50% and 54−84%, respectively.[63]

The elemental composition of the hydrolysis solids indicates a H/C ratio that is lower than that of the unreacted biomass. This result can be attained by either loss of material with a high H/C ratio (e.g., glycerol lost to the aqueous phase) or by condensation of cellular material to form more hydrogen deficient moieties. Carbon and nitrogen not retained in the hydrolysis solids were most likely liberated as aqueous species. The aqueous phase was amber with a foul odor and acidic pH (∼5). Preliminary work characterizing the aqueous product of hydrolysis reactions showed that it contained about 10−20% and 20−40% of the initial biomass COD and N, respectively. HPLC analysis revealed the presence of various hydrolysis and decomposition products, such as acetic, lactic, and citric acid, pyroglutamic acid, and glycerol. We suspect this aqueous phase can serve as a nutrient source to grow additional lipid-rich microorganisms, including *C. vulgaris*.

Supercritical in Situ Transesterification. Solids generated from the hydrolysis of wet algal biomass at 250 °C and 45 min were selected for SC-IST/E experiments. The solids from this particular reaction time were selected because they appeared to provide the best balance between high lipid retention (87%) and significant TG hydrolysis (∼67% conversion). The hydrolysis solids were not dried prior to SC-IST/E and contained approximately 46 wt % water. The ability to convert FA and the remaining glycerides in this wet solid into biodiesel through an uncatalyzed reaction with ethanol was assessed in exploratory experiments with two levels each of time, temperature, and ethanol loading (Table 2).

In general, longer reaction time and higher temperature led to higher crude biodiesel yields and significantly fewer non-FAEE components therein, particularly FA and MG. In contrast, the higher ethanol loading led to significantly greater gravimetric yields of crude biodiesel but with concomitant increases in its non-FAEE content. According to

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Table 2. Supercritical in Situ Transesterification Conditions and Crude Biodiesel Yield and Composition

reaction conditions and loading					crude biodiesel yield (%)[c]	crude biodiesel composition (wt%)					
run number	temp (°C)	time (min)	EtOH (w/w)[a]	H_2O (%)[b]		FAEE	FA	MG	DG	TG	other
1	275	60	2.2	21.0	56.4	53.5	11.7	10.1	2.1	2.1	20.5
2	275	60	8.3	8.4	80.5	39.8	14.0	16.7	6.7	2.7	20.1
3	275	120	2.3	20.4	68.3	79.2	9.3	5.3	1.7	1.3	3.1
4	275	120	7.5	9.1	94.0	56.6	9.7	13.2	2.2	0.8	17.5
5	325	60	2.1	21.6	65.8	33.4	9.9	3.2	0.8	0.6	52.2
6	325	60	7.2	9.4	87.7	52.0	13.9	10.7	0.8	0.4	22.3
7	325	120	2.3	20.4	58.7	58.6	8.6	2.0	0.6	0.1	30.1
8	325	120	6.6	10.1	100	58.7	9.0	4.3	0.3	0.1	27.6

[a] Ethanol loading is the mass ratio of ethanol to dry hydrolysis solids. [b] H_2O % is the mass percentage of water in the reactor. [c] Crude biodiesel yield is a gravimetric determination (average standard deviation for the mean of at least two gravimetric determinations was less than 6%) and is reported as a percentage of total lipids (hexane−isopropanol extraction) in the hydrolysis solids loaded on a dry basis. The crude biodiesel was analyzed directly for fatty acid ethyl esters (FAEEs) and for fatty acids (FAs), monoglycerides (MGs), diglycerides (DGs), and triglycerides (TGs) following derivatization with MSTFA.

an analysis of covariance, which determined the statistical significance of time, temperature, and ethanol loading as main predictors of crude biodiesel yield and composition using linear regression, only ethanol loading was found to significantly affect the yield. However, the influence of all predictors on the aggregated amount non-FAEE components in the crude biodiesel was significant.

In this complex, multiphase reaction system, lipids were both extracted from the hydrolysis solids and transesterified. The crude biodiesel yield, which indicates overall extraction efficiency, ranged from about 56 to 100% (relative to the total lipids in the hydrolysis solids as determined by HIP extraction). As shown in Table 2, yields from reactions lasting 120 min with an EtOH/solids mass ratio of 6.6−7.5 were between 94 and 100%, suggesting that lipid removal from the solids was nearly complete under these conditions. This result was confirmed by examining residual solids from SC-IST/E and finding the FAEE content to range from 1 to 4% on a dry weight basis. Although nearly all lipids may have been removed from the hydrolysis solids during these reactions, total FAEE yields (60−66%) indicate that transesterification was somewhat inhibited (Table 4). In the discussion that follows, the influence of key process parameters on lipid extraction and conversion is elucidated through an analysis of the biodiesel yield and composition.

Table 2 provides information about the composition of the crude biodiesel. In general, its ester content decreased with ethanol loading at 275 °C, while at 325 °C, it increased or remained the same. Ester content increased with time, though with a less pronounced effect at the higher temperature. The highest ester content (79.2%) was achieved at 275 °C, 120 min, and a low (2.3) EtOH/solids mass ratio. The ester contents reported here compare well with data reported for SC-EtOH transesterification of soybean oil in systems containing 10% water: at 275 °C, the ester content of the recovered product was approximately 10, 40, or 50% at 21, 42, or 52.5 min residence times, respectively, and 58, 69, 70.8% esters at 325 °C.[64] In addition, they compare favorably to a previous report regarding in situ transesterification of dry rice bran containing 17.4% total oil (comprising 73.7% TG and 12.3% FA): the FAME yield was 51%, and FAMEs composed 52.5% of the product mixture following the reaction with SC-MeOH and a CO_2 cosolvent (300 °C, 30 MPa, 5 min, 271 MeOH/oil molar ratio).[22] As detailed in Table 2, the crude biodiesel contained unreacted glycerides and FA in addition to esters. TGs and DGs were typically present in low amounts, especially at 325 °C. At both temperatures, increasing the amount of ethanol resulted in crude biodiesel containing a higher proportion of FA and MG. For example, in runs 4 and 8, FAEE accounted for only 57−59% of the crude biodiesel product; a significant amount of FA (9.0−9.7%) and MG (4.3−13.2%) were present. The prevalence of MGs can be explained by their increased stability relative to other glycerides, and previous work demonstrating their conversion is the rate-limiting step in TG transesterification.[15] Likewise, FA in the crude biodiesel most likely resulted from incomplete esterification or from FAEE hydrolysis. Since EN14214 mandates that esters comprise more than 96.5% of biodiesel and limits the amount of FA, MG, DG, and TG in biodiesel to 0.4, 0.8, 0.2, and 0.2 (w/w), respectively, the crude biodiesel produced by SC-IST/E under the conditions examined herein does not meet the specifications for finished biodiesel. In general, non-FAEE components could be removed with a warm alkaline water wash followed by centrifugation[65] or with new techniques that require less water.[66,67] FA and glycerides recovered from the crude biodiesel could be recycled back to the supercritical reactor, generating additional FAEE. Clearly, further work is required to explore the parameter space more fully and identify optimal conditions for producing fuel-grade biodiesel from wet, FA-rich solids via uncatalyzed, SC-EtOH reactions.

Both the ethanol and water content of SC-IST/E reactions may have impacted the yield and quality of the biodiesel produced. Water may play both beneficial and detrimental roles during supercritical transesterification. The benefit arises from low water contents increasing the conversion of glycerides to FAEE at 250−325 °C, most likely through increased glyceride hydrolysis prior to esterification and decreased decomposition of unsaturated FAEE.[64] The detriment arises from higher water contents diluting the system (thereby reducing reaction rates) and facilitating FAEE hydrolysis (loss of desired product). These phenomena are likely responsible for the reduced ester yields reported from SC-EtOH transesterification reactions done in the presence of water.[68−70] Likewise, the ethanol loading can have positive and negative impacts on FAEE yield.

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Table 3. Fatty Acid Ethyl Ester Composition of Biodiesel Produced through Supercritical in Situ Transesterification[a]

	fatty acid profile (% of total FAEE)						percent isomerized (% of total species)	
run number	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C18:2	C18:3
1	0.0	19.3	2.2	47.7	9.3	11.8	8.0	42.2
2	0.0	18.5	2.3	48.1	9.0	12.4	0.0	34.9
3	0.5	20.1	2.3	50.4	8.4	9.8	8.4	59.1
4	0.4	19.1	2.2	49.6	8.9	11.4	6.3	53.9
5	0.6	23.2	2.9	57.1	6.1	3.4	43.8	77.2
6	0.5	20.1	2.4	52.1	8.6	7.7	34.5	83.6
7	0.7	24.9	3.5	59.4	4.1	0.0	57.1	*b*
8	0.4	21.0	2.6	54.3	8.3	5.4	55.9	80.7

[a] Isomerized ethyl esters correspond to both positional and geometric isomers, which demonstrated earlier (typically *trans*) and later (typically *cis*) retention times compared to those of compounds with the same chain length and number of double bonds in unreacted algal biomass where only single peaks were detected. C18:1, C18:2, and C18:3 data include all isomers detected, with the percent isomerized indicated in the two rightmost columns. [b] No C18:3 FAEE was detected in this reaction.

The equilibrium conversion to esters increases with ethanol loading, but high EtOH/FA molar ratios (>15−30) can result in lower ester yields,[26,60] perhaps because of dilution effects that reduce reaction rates. In the SC-IST/E reactions we ran with low ethanol loading, the molar ratios of ethanol to FA, MG, DG, and TG (estimated on the basis of C18:1 FA groups) were in the ranges of 45−50, 240−260, 150−170, and 170−190, respectively. In all of the esterification reactions in Table 2, the initial water-to-oil ratio was constant (ca. 1.1 w/w water to HIP CLE of hydrolysis solids). Therefore, reactions with a higher ethanol loading necessarily had a lower water content on a total mass basis (cf. 8−10% to 20−21%). This conflation of the effects of ethanol loading and water content makes it difficult to isolate the effect of either component in our experiments, but given the large excess of water and ethanol, relatively long residence times, and the prevalence of FA in the product mixtures, it is likely that dilution of the reactant mixture and FAEE hydrolysis limited ester yields. Therefore, future work should consider lower ethanol loadings and perhaps removing some or all of the water from the hydrolysis solids. In addition to improving the ester yield from SC-IST/E, these process changes may also reduce process costs and energy inputs.

Supercritical alcohol treatment has been noted to cause transisomerization and decomposition or polymerization of unsaturated FA.[71−73] Though some have suggested that isomerization reduces fuel stability[74] and negatively impacts cold flow properties,[71] there is little conclusive evidence that *trans*-isomers of unsaturated FAEE are actually detrimental to fuel quality. Nevertheless, we estimated the extent to which isomerization occurred by examining GC retention time shifts in the elution of unsaturated FAEE. Note that both isomerization and decomposition can contribute to changes in the FAEE profile of the synthesized crude biodiesel. Since no isomerization of C18:2 was detected in the hydrolysis solids, the appearance of C18:2 isomers following exposure to SC-EtOH permits a useful analysis of the effects of time, temperature, and ethanol loading on isomerization. Table 3 shows that at 275 °C, between 6 and 8% of the C18:2 synthesized was in a non-native form, with the exception of the reaction lasting 60 min with high ethanol, in which no isomerization was detected. Significantly more C18:2 isomerized at 325 °C; between 34 and 44% and 56−57% of the C18:2 synthesized was in a non-native form after 60 or 120 min, respectively. At both temperatures, higher amounts of ethanol led to a reduction in isomerization, with a more prominent effect evident at shorter times. Similar but more pronounced trends were observed for C18:3 FAEE. Although analysis of C18:3 isomerization is complicated by the fact that the hydrolysis solids already contained some isomerized linolenic FA, about 35−60% and 77−83% of C18:3 FAEE detected in the crude biodiesel was isomerized at 275 and 325 °C, respectively. In summary, higher temperatures and longer reaction times resulted in greater transisomerization of C18:2 and C18:3 FAEE, in agreement with previous reports.[68,73]

Table 4. Fatty Acid Ethyl Ester Yields from Supercritical in Situ Transesterification[a]

	ester yield		yield reduction[c]		
run number	total	saturated[b]	C18:1	C18:2	C18:3
1	34.2	37.2	1.4	1	9.3
2	36.3	39.7	1.4	2.3	8.5
3	49.5	55.4	0.6	8	21.9
4	60.2	65.1	−0.5	3.6	17.5
5	43.0	58.4	4.4	28.5	48.2
6	51.6	59.7	0.7	8.7	32.3
7	38.9	60.6	9.9	42.3	60.6
8	66.4	81.3	2.1	18.2	56.5

[a] All yields are based on GC-FID determinations of FAEE from the acid catalyzed in situ transesterifaction of hydrolysis solids and of FAEE recovered from supercritical in situ transesterification. [b] Saturated FAEE yield is the average yield of C16:0 and C18:0 FAEEs. [c] The difference between the average yield of saturated FAEE and the yield of all isomers of C18:1, C18:2, or C18:3.

In addition to isomerization, unsaturated FA can undergo a variety of reactions that may impact their usefulness as fuel components. It has been suggested that polyunsaturated FA can polymerize into higher molecular weight compounds and decompose into gaseous products in the presence of methanol above 300 °C, thereby reducing ester yields.[71] For example, when methyl linolenate was reacted with methanol at 350 °C and 43 MPa for 20 and 40 min, total ester recovery was only 20.6% and 14.4%, respectively.[71] We examined the decomposition of unsaturated FAs during SC-EtOH treatment of the hydrolysis solids. Decomposition is indicated when the yield of all isomers of an unsaturated FAEE is less than the yield of saturated FAEEs, which are assumed to be thermally stable under these conditions.[71] We also implicitly assume that the transesterification rates in SC-EtOH are identical for both saturated and unsaturated FAs. The data in Table 4 show that decomposition was most prevalent in the polyunsaturated FAEE at higher temperatures and longer times. For example,

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the difference between the C18:3 yield and the yield of saturated FAEE increased between 3−5 and 1.2−1.5-fold from 275 to 325 °C and 60 to 120 min, respectively. In addition, yields of C18:2 and C18:3 FAEE, inclusive of all isomers, were closer to the average yield of saturated FAEE in reactions containing more ethanol, with the exception of those carried out at 275 °C and 60 min. In contrast, the yield of native C18:1 was within 2% of the average yield of saturated FAEE in all treatments, with the exception of reactions at 325 °C containing low amounts of ethanol. Here, C18:1 yields were about 4.4 and 10% less than the average yield of saturated FA at this temperature and 60 or 120 min, respectively. These data suggest that thermal reactions consume unsaturated FAEE, generating compounds that were not detected in this study.

Some of these products, such as glycerol decomposition products and short chain FAEE, may still contribute to the biodiesel as fuel components and may even improve the biodiesel viscosity and cloud/pour point.[75] Recall from Table 2 that in most reactions about 20−30% of the gravimetric mass of the crude biodiesel remained unidentified by GC-FID. This result is consistent with the findings of Kasim et al.,[22] who suggested that unidentified matter was most likely the degradation products of proteins, carbohydrates, and hydrocarbons. While biodiesel may be narrowly defined by international specifications to contain only long-chain monoalkyl esters, we suggest that the total fuel yield from SC reactions may contain non-FAEE components that are still valuable. In addition, exposing feedstocks rich in polyunsaturated FA to SC-EtOH may be a useful way to generate biodiesel that meets EN14103 specifications (i.e., C18:3 content < 15% of esters).

In general, the FAEE yield from both SC oil transesterification and FA esterification tends to increase with residence time until a critical point, after which it decreases. The competing phenomena of conversion and decomposition define this optimum time, which tends to occur earlier at higher temperatures.[73] Since isomerization and thermal decomposition were less pronounced at 275 °C, the longer reaction time increased crude biodiesel and FAEE yields. At 325 °C, increasing the reaction time from 60 to 120 min decreased both crude biodiesel and FAEE yields at the lower ethanol loading but increased yields at the higher ethanol loading. These data highlight how the decomposition of unsaturated FAEE, evidenced in part by the complete absence of C18:3 FAEE in run 7, can influence fuel yields when considering esters only. Since data in the factorial experiment was collected at only two time points, little can be inferred about whether a maximum in yield occurred at intermediate times. However, in additional SC-IST/E experiments carried out for 60, 90, and 120 min at 290 °C, crude biodiesel and FAEE yields were highest at 90 min by 10−20%. Taken together, these data suggest that reaction time must be chosen wisely to optimize conversion and limit decomposition.

Finally, residual solids remaining after SC-IST/E were found to contain approximately 68−72% C, 5.6% H, and 3.1% N. We suspect these defatted solids can be used as a soil amendment, providing both fertilizer value and enhancing soil carbon content and microbial activity as has been demonstrated for other biochars.[76]

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Conclusions

We have demonstrated the feasibility of a two-step hydrolysis−solvolysis process to produce biodiesel from lipid-rich, wet algal biomass. This process obviates biomass drying, organic solvent extraction, and catalysts, while providing a mechanism for nutrient recycling. A cursory investigation of the influence of some key process variables led to crude biodiesel and FAEE yields as high as 100 and 66%, respectively, on the basis of lipids within the hydrolysis solids. Considering that about 80−90% of lipids in the original algal biomass were retained in the solids recovered after hydrolysis, the total process yield was somewhat lower. The optimal time and temperature for hydrolysis must appropriately balance the desire for increased lipid hydrolysis with the likelihood of reduced lipid retention and solids yields at more severe conditions. In addition, it is imperative to improve the ester yield from SC-IST/E, which may have been limited by incomplete transesterification, decomposition/polymerization of unsaturated FA, hydrolysis of FAEE, or incomplete lipid extraction from the solid. More remains to be understood regarding how whole cells, hydrothermally processed algal biomass, and intracellular constituents influence SC-IST/E and potentially contribute to nonester components in the final fuel product. Additional research and process optimization are likely to improve yields and reduce process inputs (e.g., ethanol), thereby minimizing the overall environmental impact of algal biodiesel production. To be economically viable, biodiesel yields must be above 95% and preferably higher than current norms achieved with alkali-catalyzed processes (~97%).[77]

A considerable benefit of the process described herein is the ability of its first step (hydrolysis) to create two sterile products: a relatively low moisture (< 50% water), FA-rich solid and a nutrient-rich aqueous phase. Both are potentially amenable to a variety of downstream processes. Considering that the N and P required for producing algal biomass are nonrenewable resources, the ability to recycle these nutrients, together with a useful carbon source like glycerol, presents unique opportunities to further reduce the impact of algal biodiesel production. This approach is particularly attractive if algal biomass is grown in wastewater since pathogens and competitive organisms can be destroyed prior to reusing the nutrients in algal cultivation. With regards to the hydrolysis solids, we have chosen to highlight the benefits of SC-IST/E as one possible conversion strategy for the production of biodiesel. This process is rapid, does not require catalysts, simplifies product purification, and produces another sterile biosolid. However, other options also exist. It may be beneficial to react hydrolysis solids (both wet or dry) using acid-catalyzed in situ esterification or convert FA and unconverted MG, DG, and TG in the wet hydrolysis solids to hydrocarbon biofuels via hydrothermal catalytic decarboxylation.[78,79]

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