COLLECTION AND CONVERSION OF ALGAL LIPID

by

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STATEMENT OF DISSERTATION APPROVAL

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ABSTRACT

Sustainable economic activities mandate a significant replacement of fossil energy by renewable forms. Algae-derived biofuels are increasingly seen as an alternative source of energy with potential to supplement the world's ever increasing demand. Our primary objective is, once the algae were cultivated, to eliminate or make more efficient energy-intensive processing steps of collection, drying, grinding, and solvent extraction prior to conversion. To overcome the processing barrier, we propose to streamline from cultivated algae to biodiesel via algal biomass collection by sand filtration, cell rupturing with ozone, and immediate transesterification. To collect the algal biomass, the specific *Chlorococcum aquaticum* suspension was acidified to pH 3.3 to promote agglomeration prior to sand filtration. The algae-loaded filter bed was drained of free water and added with methanol and ozonated for 2 min to rupture cell membrane to accelerate release of the cellular contents. The methanol solution now containing the dissolved lipid product was collected by draining, while the filter bed was regenerated by further ozonation when needed. The results showed 95% collection of the algal biomass from the suspension and a 16% yield of lipid from the algae, as well as restoration of filtration velocity of the sand bed via ozonation. The results further showed increased lipid yield upon cell rupturing and transesterified products composed entirely of fatty acid methyl ester (FAME) compounds, demonstrating that the rupture and transesterification processes could proceed consecutively in the same medium, requiring no separate steps of drying,

extraction, and conversion. The FAME products from algae without exposure to ozone were mainly of 16 to 18 carbons containing up to 3 double bonds, while those from algae having been ozonated were smaller, highly saturated hydrocarbons. The new technique streamlines individual steps from cultivated algal lipid to transesterified products and represents an improvement over existing energy-intensive steps.

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CHAPTER 1

INTRODUCTION

As greenhouse gas increases, environmental sustainability mandates a significant source of carbon-neutral, renewable forms of energy; yet presently such a source to replace fossil fuels accounts for only 2.1% in global energy consumption (Amin, 2009; BP, 2012). Algae-derived biofuel is renewable, biodegradable, and environmentally benign (Ahmad et al., 2011); it is seen with great potential to fulfill global demand for transportation fuels (Chisti, 2007; Schenk et al., 2008; Demirbas and Demirbas, 2011).

Biodiesel production from microalgae involves cultivation, collection, dewatering, lipid extraction, and lipid transesterification. While many advances have been made on the selection and cultivation of suitable algal strains and the transesterification has been well established in recent decades, the collection, dewatering, and extraction of algae for lipid remain the bottleneck requiring energy-intensive processes and combined processes such as flocculation, sedimentation, centrifugation, filtration, flotation, and electrophoresis (Schenk et al., 2008). No single process is viewed as technically and economically superior for harvesting and dewatering (Uduman et al., 2010). The technical and economical efficiencies of the processes depend highly on the algal species, size, and density (Uduman et al., 2010; Brennan and Owende, 2010; Abdelaziz et al., 2013). As a wet biomass decreases conversion efficiency (Johnson and Wen, 2009),

dewatering presents an additional challenge (Danguah et al., 2009; Uduman et al., 2010). However, traditional dewatering methods such as heating and freeze drying are energy intensive and almost inapplicable in practice.

Sand filtration has been widely practiced for removal of suspended solids from water for many decades. Without chemical addition, *Scenedesmus quadricauda* were entrapped in fine sand/silt with 97% removal rate (Naghavi and Malone, 1985). By fine sand filtration with or without pretreatment, microalgae can potentially be entrapped while backwashing of the sand filter allows concentration and reuse of the sand filter.

Ozone is a powerful oxidant and disinfectant that has been widely used in water and wastewater treatment (Yukselen et al., 2006). Ozonation not only removes algal toxins and oxidizes micropollutants (Boisdon et al., 1994; Rositano et al., 2001; Hoeger et al., 2002), but also promotes cell wall rupture, releasing intracellular matter into the liquid medium (Albuquerque et al., 2008; Miao et al., 2009). For concentrated algae with limited contact between ozone and algae, long ozonation time may be necessary, which consumes a large amount of energy. Our prior research showed ozonation via compression and decompression cycles in succession as a means to deliver ozone increased cell rupture efficiency even at a smaller ozone dose and shorter contact time (Cheng et al., 2012).

In this study, a new processing scheme that combines sand filtration and ozonation for the collection and extraction of algal lipid is developed. The process streamlines necessary steps of chemical coagulation, filtration, dewatering, mechanical grinding, and solvent extraction – a series of energy and cost intensive steps that presents a great challenge in procuring algal biofuel today. Increasing the collection efficiency of

sand filtration via different pretreatments is the first step. After the algae is collected successfully, ozonation will be used to rupture the cell wall of algae to release its lipid into the liquid phase. Then, I extended the process to streamline the production of biodiesel compounds, and investigated rupturing of the collected wet algae by ozone followed by transesterification of the dissolved lipid. The focus is on eliminating the drying and solvent extraction steps as shown in Figure 1.1 and on the resulting products from the streamlined process under varied conditions. The final phase of this research was to design an integrated system of filtration and ozonation in a single vessel, where algae collection, algae rupturing, and lipid recovery can be accomplished in the single vessel and immediately followed by conversion into biodiesel.

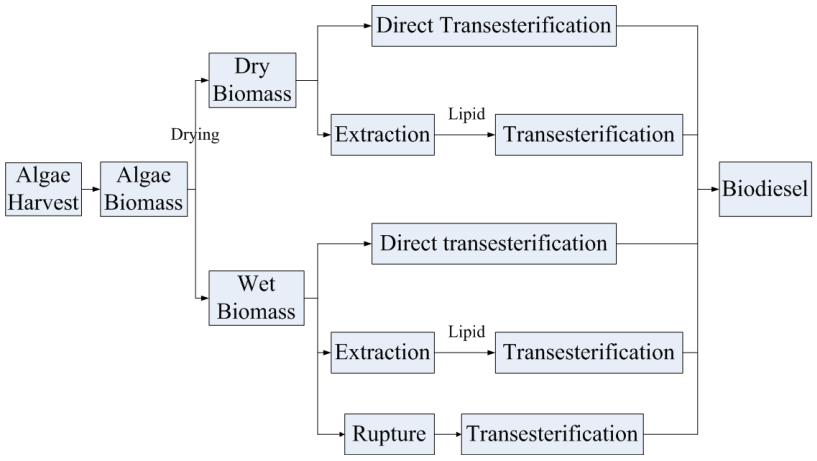


Figure 1.1 Different harvest and conversion steps to produce biodiesel

CHAPTER 2

LITERATURE REVIEW

2.1 Energy problem

World primary energy including coal, renewable, hydroelectricity, nuclear energy, natural gas, and oil, consumption grew by 2.5% in 2011, roughly in line with the past 10-year average, and the annual world primary energy consumption was estimated at 12,274 million tons of oil equivalent (mtoe). Fossil fuels accounted for 86.9% of the primary energy consumption, with oil (33.1% share), coal (30.1%) and natural gas (23.7%) as the major fuels (BP, 2012). It is widely accepted that the use of fossil fuels has caused global warming; therefore major fuels as a source of energy need to be replaced with renewable, clean energy sources in order to reduce carbon dioxide and greenhouse gas emissions (Amin, 2009); However, the share of renewables in global energy consumption is only 2.1% according to BP's annual report (BP, 2012).

2.2 Potential role of biofuels from microalgae

Microalgae, as biomass, are a potential source of renewable energy, and they can be converted to biodiesel, bioethanol, bio-oil, biohydrogen and biomethane via thermochemical (gasification, pyrolysis, liquefaction, and hydrogenation) and biochemical (fermentation and transesterification) methods as identified in Figure 2.1

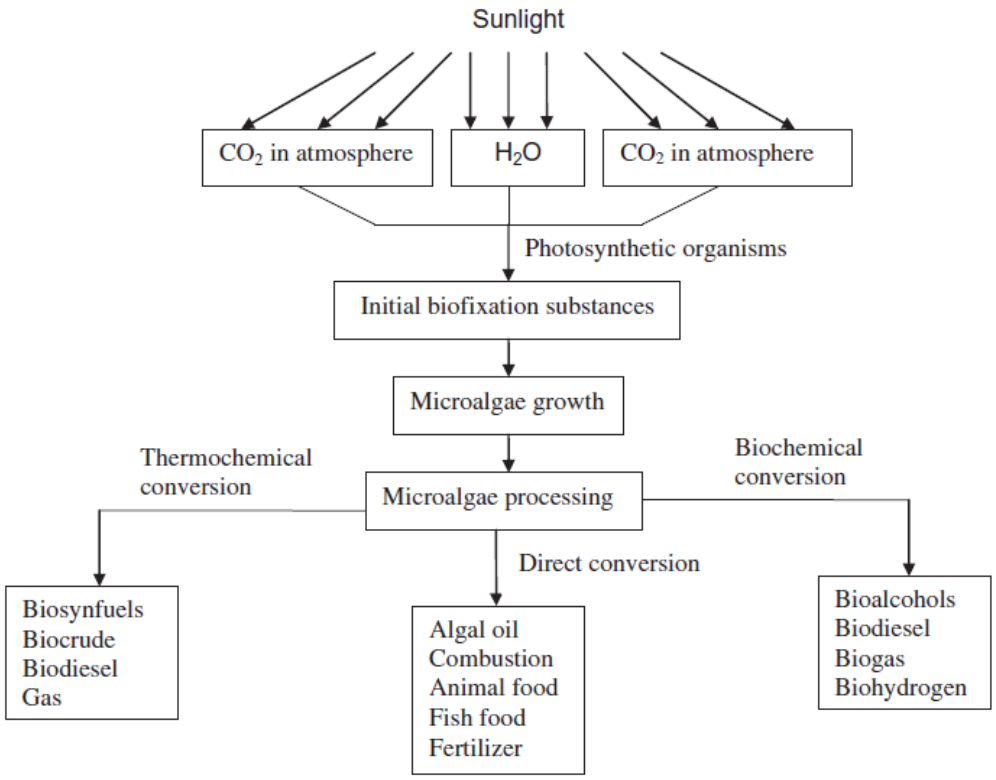


Figure 2.1 Carbon dioxide fixation and main steps of algal biomass technologies (based on Demirbas, 2011)

(Amin, 2009; Brennan and Owende, 2010; Demirbas, 2011). The main advantages of microalgae-derived biofuels (oilgae or third generation biofuel) are: (1) High growth rate, microalgae are capable of year-round production with higher oil productivity (high oil content 20-50% dry weight of biomass) than the yield of the best oilseed crops; (2) less water demand than land crops as they grow in either freshwater or brackish water; (3) high-efficiency CO_2 mitigation as 1 kg of dry algal biomass utilize about 1.83 kg of CO_2 (Chisti, 2007); and (4) more cost-effective farming as nutrients for microalgae cultivation (especially nitrogen and phosphorus) can obtained from wastewater without requiring herbicides or pesticide in cultivation (Brennan and Owende, 2010; Demirbas, 2010).

2.3 Algal biodiesel

Biodiesel is an alternative biofuel produced by chemically reacting a vegetable oil or animal fat with a short-chain alcohol, such as methanol, ethanol, or buthanol in the presence of a catalyst (Meher et al., 2006). Biodiesel is the main alternative to fossil fuel because it is sustainably supplied, highly biodegradable, and environmentally friendly. It also has advantages such as low emission from combustion, no contribution to global warming because of its closed carbon cycle, good performance for existing engines, and increased energy security among others. Moreover, microalgae appear to be the only source that can be sustainably developed in the future (Ahmad et al., 2011) and are seen as capable of meeting global demand for transportation fuels (Chisti, 2007; Schenk et al., 2008; Demirbas, 2011). Thus, the use of algae as feedstock for biodiesel production is rapidly growing in the United States and the world (Brentner et al., 2011).

2.4 Oil content of algae

Oil content in microalgae is commonly around 20%-50%, but it can exceed 80% by weight of dry biomass (Metting, 1996; Spolaored et al., 2006). Oil productivity, the mass of oil produced per unit volume of the microalgal broth per day, varies depending on the algal growth rate and the oil content of the biomass (Chisti, 2007). In general, the growth rate and lipid content were inversely related. Rodolifi et al. (2008) found among the best producers in terms of biomass or lipid production rates in *Cholorococcum sp.*, *Scenedesmus sp.*, and *Cholorella sp.* at 53.7, 53.9, and 42.1 mg Lipid/L/day, respectively, in freshwater and in *Nannochloropsis sp.*, and *T. suecica* at 61.0, and 36.4 mg Lipid/L/day, respectively, in marine. Table 2.1 shows the yields of various plant oils,

Table 2.1 Yield of various plant oils (Demirbas et al., 2011)

Crop	Oil in liters per hectare
Algae	1,00,000
Castor	1413
Coconut	2689
Palm	5950
Safflower	799
Soy	446
Sunflower	952

which shows algal oil as a promising source of biodiesel.

2.5 Cultivation of algae

There are two types of cultivation reactors for algae, open systems and closed photobioreactors. Raceways (open systems) are perceived to be less expensive to build and operate. The main disadvantage is that evaporation and contamination by unwanted species easily occur as it is open to the atmosphere. Closed photobioreactors including plate, tubular, annular, and plate airlift, provide much greater oil yield per hectare compared with raceway bond, because the volumetric biomass productivity of photobioreactors is more than 13-fold greater in comparison with raceway ponds (Chisti, 2007; Schenk et al., 2008).

The reactor type is an important factor for cultivation, but other factors can also affect algal biomass productivity such as culturing conditions including temperature, mixing, fluid dynamics and hydrodynamic stress, gas bubble size and distribution, gas exchange, mass transfer, light cycle and intensity, water quality, pH, salinity, anmineral and carbon regulation/bioavailability, cell fragility, cell density, and growth inhibition (Schenk et al., 2008).

Algae cultivation can also be conveniently placed in a wastewater treatment plant. Algal reactors remove 19% of dissolved nitrogen and 43% of dissolved phosphorus from wastewater effluents (Sturm et al., 2012). Industrial and municipal wastewaters are potential resources for production of biofuels from microalgae (Chinnasamy et al., 2010; Kim et al., 2010; Xin et al., 2010).

2.6 Harvest method

From microalgae biomass for biodiesel production, the traditional processes include algal strain selection, cultivation, harvest with dewater, extraction, conversion (transesterification), and purification, as in Figure 2.2.

Currently, the cost of harvesting algae from the growth medium is critical (Parkavi et al., 2010). Techniques that result in greater algal biomass may have drawbacks such as high capital cost or energy consumption (Uduman et al., 2010).

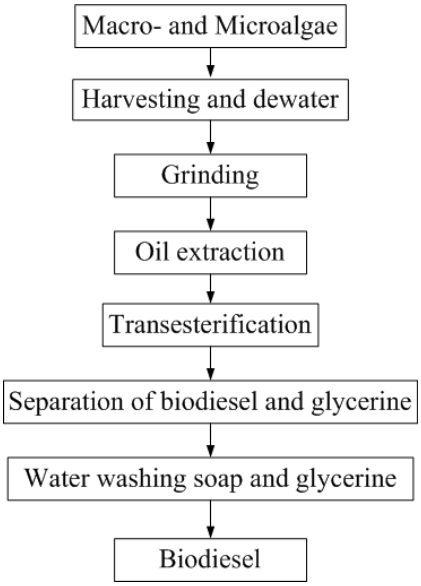


Figure 2.2 Traditional processing for production of biodiesel from algae

Flocculation, microscreening, and centrifugation are the most common harvesting processes (Schenk et al., 2008). All of them require high energy input. For example, microscreen such as membrane can provide good separation efficiency (Danquah et al., 2008; Petruševski et al., 1995), but high maintenance costs are often required to prevent membrane-clogging problems. Centrifugation also can separate water and algae effectively, but it can only treat a small volume considering its high energy expense. Flocculation concentrates algae readily in small scale, but it is too expensive for large-scale operations and the algal chemical sludge cannot be used for some downstream applications (Danquah et al., 2008). Besides, after harvesting and dewatering, the algal biomass (now of 5-15 % dry mass) must be processed rapidly or be subjected to deterioration in hours under warm climate. Drying methods include spray drying, drum drying, free-drying, and sun drying, which incur significant time and cost. In some cases, solvent extraction of the wet biomass has proved less effective for recovery of the cellular material than extraction of the dry biomass (Grima et al., 2003; Brennan and Owende, 2010). In addition, the biodiesel yield and fatty acid methyl ester (FAME) contents from direct transesterification of a wet biomass were significantly lower than those from dry biomass (Johnson and Wen, 2009), suggesting the need for drying the collected algae. After drying, the algal lipid needs to be extracted by an organic solvent, expeller/oil press, supercritical fluid, or under ultrasonic irradiation (Abdelaziz et al., 2013), with varying effectiveness, cost, and sustainability implications. An ideal extraction process should favorably extract the lipid fraction (neutral lipids containing mono-, di-, and trienoic fatty acid chains) minimizing nonlipid contaminants (Halim et al., 2011).

2.7 Transesterification

Transesterification is the process of exchanging the alkoxy group of an ester compound by another alcoholic molecule to form glycerol and methyl esters (biodiesel, FAME). These reactions are often catalyzed by base or acid (Demirbas, 2007). Methanol is used in this process, i.e., methanolysis. Methanolysis of triglyceride is the key reaction for algal biodiesel production, as shown in Figure 2.3.

The transesterification process is affected by reaction conditions, such as the molar ratio of alcohol to oil (3-15:1), type of alcohol (methanol or ethanol), type (alkali, acid, enzyme, or heterogeneous catalysts) and amount of catalysts (0.5-2.25 M), reaction time (1 min to 1 h), temperature (30-60 ^{0}C), and the purity of reactants (Meher et al., 2006).

2.8 Sand filtration for algal harvesting

In the water treatment arena, sand filtration is common as a unit operation to remove contaminants and it has been used for decades. Conventional filtration operated under pressure or vacuum has been successfully used to recover relatively large microalgae (>70 μm) such as *Coelastrum proboscideum* and *Spirulina platensis.* However, It becomes difficult to retain organisms approaching bacterial dimensions

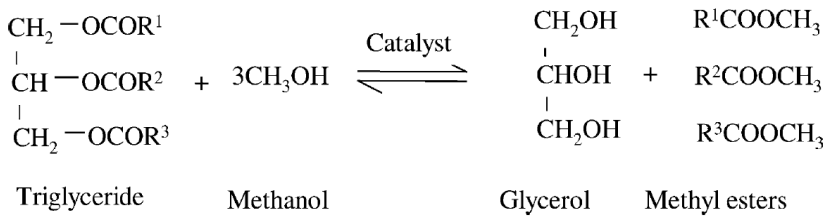


Figure 2.3 Chemical equation for transesterification of triglycerides

(e.g., <30 μm) such as *Scenedesmus, Dunaliella, Chlorella* (Uduman et al., 2010; Brennan and Owende, 2010, Grima et al., 2003).A concentration factor of 245 times the original concentration for *Coelastrum proboscideum* was achieved with a 27% solid sludge by filtration processes (Mohn, 1980). Naghavi et al. determined the potential of filtering algae from water using fine sand/silt as a filter medium (0.064-0.335 mm). Without chemical addition, the average removal of algae (*Scenedesmus quadricauda*) from water was 97.3% with low average initial head loss across the filter medium of 7.3 cm (Naghavi et al., 1986). These early reports have demonstrated feasibility of removing algae via sand filtration. A recent review on dewatering of algal biomass showed only minimal evidence on the use of sand filtration for algae harvesting (Lin and Hong, 2013); the challenge remains that only large algal particles could be economically harvested.

2.9 Ozonation

Absent prolonged contact of the biomass with solvent, induced cell disruption is seen as beneficial in expediting the recovery of lipid from microalgae. Physical methods such as autoclave and mechanical disruption in a high-pressure homogenizer as well as chemical methods such as with acid, alkali, and enzymes are effective in making the cell contents accessible (Mendes-Pinto et al., 2001; Grima et al., 2003). Ozone, a common oxidant and disinfectant for water and wastewater treatment usually employed as the first and/or an intermediate oxidation step and in many cases also as a final disinfection procedure (von Gunten, 2003), has been used to disrupt biomasses including activated sludge and algae (Yukselen et al., 2006; Cheng et al., 2012; Huang et al., 2014). In this work, ozone as a potent oxidant is employed to rupture the algal cell wall to accelerate

the release of lipid into the liquid medium. Miao et al. showed that ozone was capable of damaging the cell wall of *Microcystis aeruginosa* algae resulting in the release of cellular cytoplasm into the medium as measured by volatile organic compounds (Miao et al., 2009). The ozonation of algal cells resulted in substantial increases of assimilable and dissolved organic carbons in the water phase. Algae were not completely destroyed during ozonation, but rather shrank and released organic carbon into the water (Albuquerque et al., 2008). Therefore, ozone not only has the potential to disrupt the protective cell enclosure (Cheng et al., 2011; Cheng and Hong, 2013), but also to remove algal toxins and micropollutants. To this purpose, a recently developed ozonation method involving consecutive cycles of compression and decompression (Hong, 2008d) has been used in this study to increase the efficiency of rupture and release of cellular materials.

In the production of biofuel from algae, harvesting and dewatering are a major bottleneck because of practicality, cost, and energy consumption. In this study, conventional, economical techniques of sand filtration and ozonation have been adopted to harvest and rupture algae to obtain the lipid content and concentrate it into a small volume. The sand filtration process is made more effective via agglomeration of the algae that increases the algae size, which is collected via gravity flow without requiring energy input. After collection, ozonation is delivered via pressure cycles to the collected algae immersed in methanol in the sand bed. The combined processes eliminate the need for drying and another extraction step, enabling the released lipid for immediate transesterificaion into biodiesel. In the present study, the only significant energy expenditure is during ozonation. Thus, this study tests a streamlining approach that

combines proven, economical processes to solve a bottleneck in the production of biofuel from algae.

CHAPTER 3

APPROACH AND METHODS

3.1 Research objectives and hypotheses

This research will test component processes with the potential of integration into a practicable, streamlined process for biofuel production from algae. The main goal is to eliminate multiple energy-intensive steps such as harvest, drying, and extraction before lipid conversion. To evaluate the potential for an implementable integrated process, this research has tested the following hypotheses and component processes in two phases and arrived at a processing design for biodiesel production:

3.1.1 Phase I- Collection of algae

Hypotheses:

1. Algae coagulate and increase in size when their surface charge is neutralized by pH adjustment.
2. Coagulated algae after size augmentation are amenable to collection by conventional sand filtration.

Tasks:

1. Investigated the size change of algae in different pH.
2. Evaluated sand filtration and recovery efficiency under pretreatments at different pH.

3.1.2 Phase II- Rupture of algae and conversion of ruptured algae

Hypotheses:

1. Ozonation causes rupturing of algae, resulting in release of algal lipid.
2. Algal lipid from ozone-ruptured cells is transesterified with less impurity.

Tasks:

1. Determined the effectiveness according to ozonation operation time and type (conventional bubbling ozonation or pressure cycles-assisted ozonation; in water or methanol) as measured by solids, COD, and lipid content.
2. Determined effective ozone dosage and transesterification efficiency by comparisons of results from Soxhlet extraction and ozone rupturing based on collected lipid and FAME amounts.

3.1.3 Phase III- Integrated engineering design and required energy analysis

Hypothesis: Algae harvesting, lipid collection, and transesterification can be combined in a streamlined design.

Tasks:

1. Integrate sand filtration and ozonation in one vessel to collect and rupture algae in Methanol, and convert into biodiesel in second vessel by optimized operation based on results of Phase I and II.
2. Designed a streamlined, integrated system for pilot testing based on results of Phases I and II.

3.2 Experimental methods and design

3.2.1 Algae

Chlorococcum aquaticum (UTEX: 2222) from The Culture Collection of Algae (University of Texas, Austin) was used for its rapid growth (Danquah et al., 2009) and wide temperature tolerance (Halim et al., 2011); it is a top lipid producer at 54 mg lipid/L/day (Rodolfi et al., 2008). It was cultivated in the laboratory in a 60-gal aquarium at room temperature of 25±2 ℃. A modified Bristol medium was used to provide essential nutrients for the algae, which contained $NaNO_3$ (2.94 mM), $CaCl_2$-$2H_2O$ (0.17 mM), $MgSO_4$-$7H_2O$ (0.3 mM), K_2HPO_4 (0.43 mM), KH_2PO_4 (1.29 mM), and NaCl (0.43 mM). Illumination was by placing above the aquarium a T5 high-output light fixture housing four 48-in fluorescent tubes totaling 216 Watts (*Sun blaze*).

For determining optimal rupturing efficiency section, when the cultivated batch reached a volatile suspended solid concentration (VSS) of 100 mg/L, one liter of the suspension was vacuum-filtered (1.6 μm, Grade GF/A Glass Microfiber Filters, Whatman) to obtain the wet algae. The wet algae were directly used, or dried in a porcelain dish in oven at 60 ^{o}C for 24 h to obtain the dry algae. For direct extraction, the wet algae on filter were transferred into a cellulose extraction thimble (33 mm × 94 mm I.D. × H, Whatman); alternatively, the dried algae were ground to powder and transferred into the extraction thimble.

3.2.2 Phase I

3.2.2.1 Pretreatment

The algal suspensions were pretreated by ozonation (contact at 150 mg O_3/g TSS), ozonation followed by pH adjustment (contact at 150 mg O_3/g TSS, then pH adjusted to 11.7 or 3.6), or only pH adjustment (to pH 3.3) to examine any changes in particle size. The adjustment of pH was via manual addition of 2 M of H_2SO_4 or NaOH solution.

3.2.2.2 Sand filtration

Sand filtration was used for collection of pretreated algal suspensions. The sand filter was constructed of a polycarbonate column of 19 cm in diameter and 38 cm in height packed with 6 layers of sieved sands increasing in size with depth: $\leqq$53 μm (thickness of 2 cm at the top), 53–250 μm (4 cm), 250–430 μm (4.5 cm), 430 μm–1.2 mm (3 cm), 1.2–2.0 mm (3 cm), and 2.0–20 mm (4.5 cm). The bed depth and area were of 21 cm and 270 cm^2, respectively. Various volumes of algal suspensions (e.g., 4 L of pretreated sample and 50 L of ozonated sample) were added at the column top, and the effluents collected at the column bottom. Backwashing was through a reversed flow of distilled water. During filtration, a constant hydraulic head (via constant height of water standing above the sand surface) was maintained by a siphon. The filtration velocity was tracked throughout.

3.2.3 Phase II

3.2.3.1 Ozone treatment for determining optimal rupturing efficiency

Algal suspensions were obtained from consecutive steps of cultivation in tank, pretreatment and collection by sand filtration, and backwashing from the sand bed. Ozonation treatment was performed in two different modes. In one mode, the suspensions were ozonated through conventional bubbling of ozone gas into the 1-L suspension in an open Erlenmeyer flask; in another mode, ozone was delivered via successive cycles of compression and decompression of the ozone gas into the 1-L algal suspension in a closed, pressure-resistant, stainless-steel reactor of 1.5 L at room temperature (25 ± 2 ℃) (Hong et al., 2008; Cheng et al., 2012). The pressure reactor as Figure 3.1 featured a gas vent and a pressure gauge at the top, inlet and outlet at the bottom, and a magnetically coupled stirrer. The reactor was loaded with an algae suspension. A pressure cycle began with the compression stage when the inlet valve was opened to admit an O_3/air mixture driven by a compressor (GAST) at a desired flowrate. The gas passed through a diffuser plate at the reactor bottom and through the liquid to pressurize the closed headspace to reach 150 psi; once reaching it, the pressure was rapidly released by opening the outlet solenoid valve at the reactor top. The time it took for the reactor to reach the designated pressure depended on the headspace volume and gas flowrate (e.g., reaching 150 psi in 10 s at 2 L min^{-1}); decompression time varied with venting speed but was typically controlled at 2–3 s. The compression-decompression cycle was repeated multiple times. Alternatively, rupturing of algae collected in the open sand bed was by addition of methanol followed by bubbling of ozone at the column bottom. Ozone gas was generated at 1.5% (v/v) at 2 L/min by an ozone generator (Model

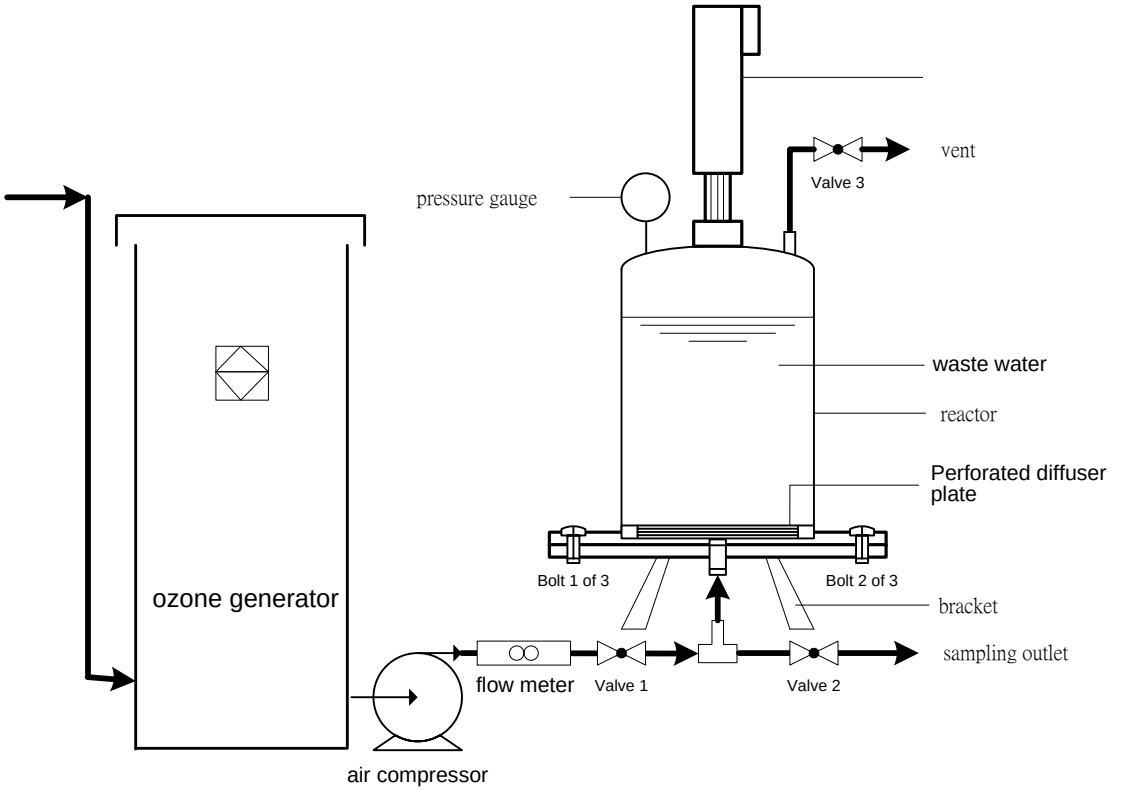


Figure 3.1 Pressure-assisted ozonation reactor

T-816, Polymetrics) being fed with dry, filtered oxygen operated at 105 V; the O_3 concentration was measured by an Indigo colorimetric method (Bader and Hoigné, 1982).

3.2.3.2 Ozone treatment for determining optimal converting efficiency

Figure 3.2 describes the experimental procedures. Filtered, wet algae were placed in a 12-mL glass vial along with 5 mL of CH_3OH. This algae/CH_3OH suspension was sparged with a 1.5% (v/v) O_3 gas stream at 1 L/min for various durations (0, 1, 3, 5, and 10 min).The O_3 concentration in the gas stream was determined by the Indigo blue colorimetric method (Bader and Hoigné, 1982). The total contact time of biomass with CH_3OH for all experiments was kept at 1 h, including varied durations of ozonation.

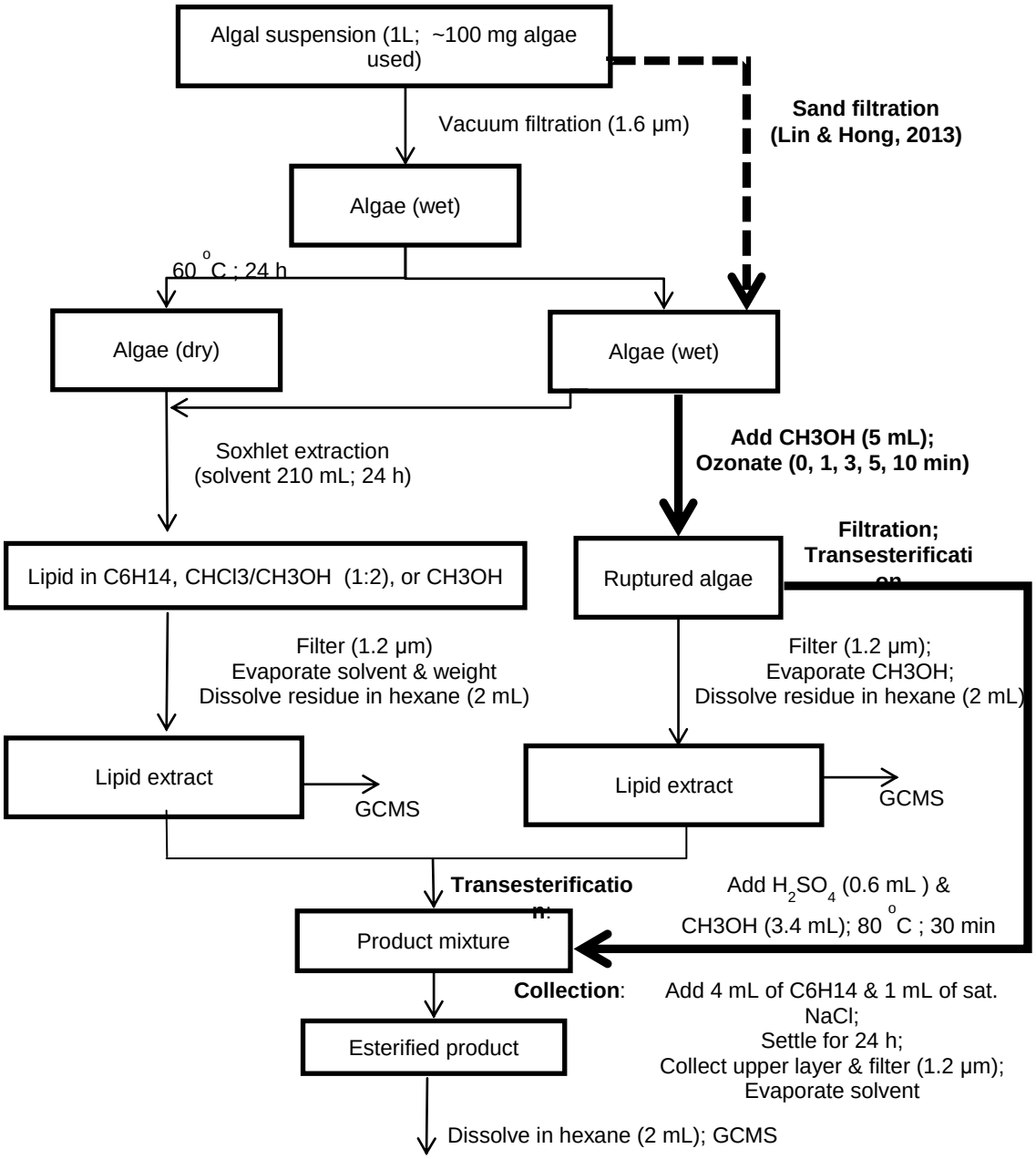


Figure 3.2 Experimental schematics, with proposed streamlined processes in bold

After ozonation the solids were separated by filtration (1.2 μm, Glass Microfibre Filter 693, VWR) fitted on a glass syringe. The filtrate was collected in a preweighed vial. The methanol was then evaporated under a gentle N_2 stream, and the residual was determined gravimetrically. The residue was dissolved again in 2 mL of n-hexane for GC-MS analysis.

3.2.3.3 Soxhlet extraction

Either wet algae or dried algal powder was put in a thimble for Soxhlet extraction for 24 h with 210 mL of either n-hexane (n-C_6H_{14}), methanol (CH_3OH)/chloroform ($CHCl_3$)(2:1 v/v), or methanol as the solvent. Afterward, the solvent was evaporated to about 10 mL using a water bath at 80 ^oC and atmospheric pressure. The remaining solution was filtered (1.2 μm, Glass Microfibre Filter, 693, VWR) by use of a glass syringe, and transferred into a clean, preweighed, 12-mL glass vial. The solvent was further evaporated by a N_2 stream; the residual was gravimetrically measured and taken as collected lipid. The residual was dissolved again in 2 mL of n-hexane for analysis by GC-MS.

3.2.3.4 Transesterification

The lipid collected after Soxhlet extraction or ozonation was placed in a vial, into which 0.6 mL of 2-M H_2SO_4 and 3.4 mL of CH_3OH were added. The mixture was maintained at 80 ^oC for 30 min. After reaction and cooling to room temperature, it was amended with 4 mL of n-hexane and 1 mL of NaCl-saturated solution. It was mixed by inverting the tube several times and then allowed to sit for 24 h. The top n-hexane layer

containing the FAMEs was collected with a glass syringe and filtered (1.2 μm, Glass Microfibre 693, VWR). After evaporation of n-hexane, the residue was gravimetrically determined as transesterified product. The product was dissolved in 2 mL of n-hexane with an internal standard (heneicosanoic acid methyl ester, C21:0) for GC-MS analysis.

3.2.4 Phase III

3.2.4.1 Integrated process design with sand filtration, rupture, and extraction in one vessel

After individual steps of pretreatment, sand filtration, and ozonation were tested, their combined operation in one vessel was performed. Figure 3.3 shows a new processing scheme from the cultivated algal suspension to procured lipid in one vessel. A smaller sand filtration column of 7 cm in diameter and 15 cm in height was used to test the integral process: filtration of algae, ozonation rupture of cells, and solvent extraction of lipid in the same vessel. The sand bed was of 6.5 cm in depth and 40 cm^2 in filtration area, packed with 4 layers of sieved sands (from top): ≤53 μm (1.5 cm), 53–150 μm (2 cm), 150-250 μm (1.5 cm), and 250–425 μm (1.5 cm). Algae suspensions and distilled water (500 mL in each run) were passed through the column; distilled water was passed through the column before and after each filtration run to determine filtration velocity resulting from increasing head loss. A constant hydraulic head (7.5 cm above the sand surface) was maintained as long as possible during the runs, and the filtration velocity tracked. After filtration that entrapped algae in the sand bed, the drained bed was added with 90 mL of methanol (solvent height reaching 2 cm above the sand surface) and ozone gas (1.5% O_3) was introduced from the bed bottom at 2 L/min for 2 min. After ozonation,

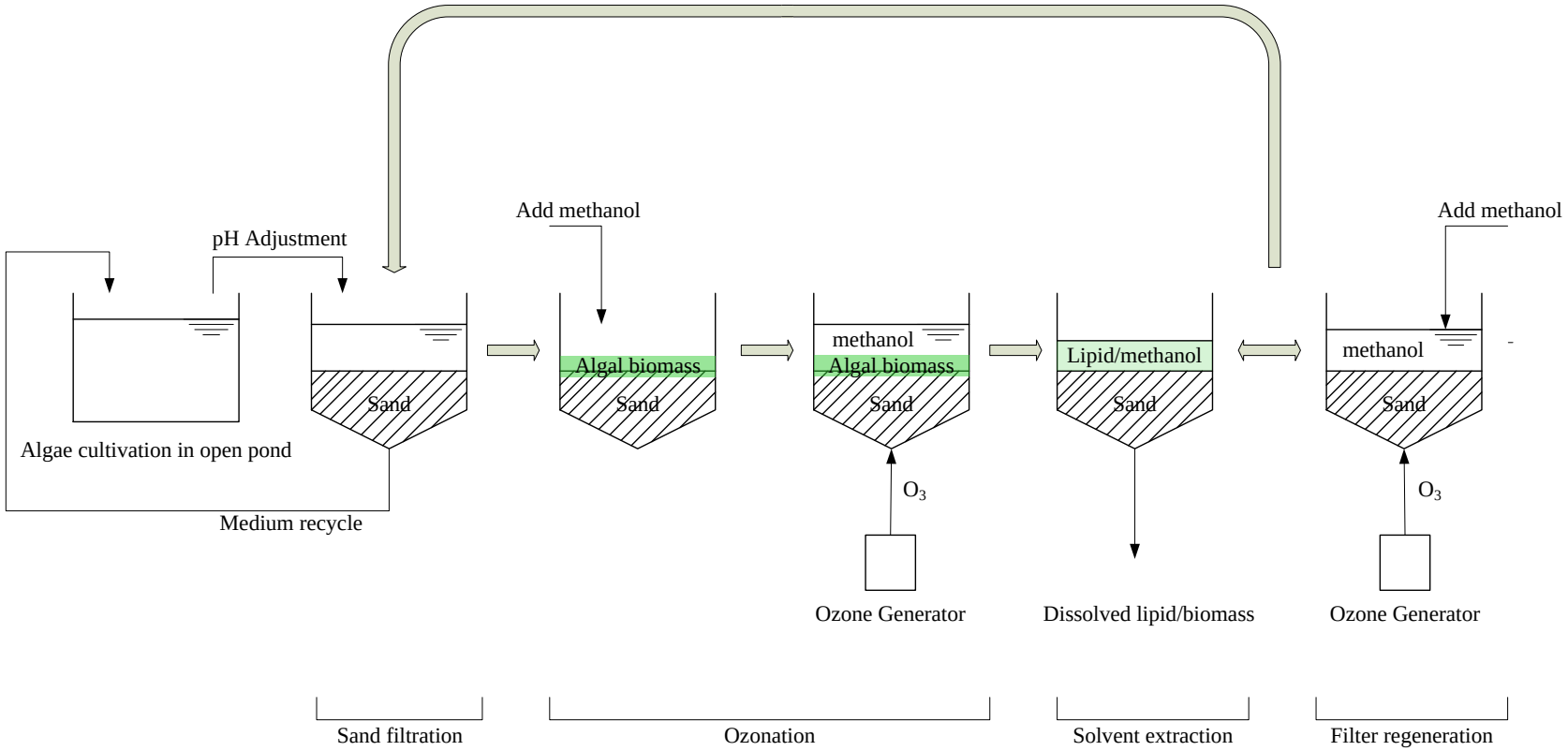


Figure 3.3 Processing scheme from cultivated suspension to algal lipid

cell contents including the lipid were released from ruptured algae into the methanol, and the solution was collected by draining. It should be noted that for simplicity reasons at this development stage of the single filtration-rupture vessel, ozonation was carried out by purging in continuous mode, and operation via pressure cycles were not used for this single vessel.

3.2.4.2 Regeneration of sand filter

The sand filter for pretreated algae was readily regenerated at the end of each filtration cycle (designated at 4 or 50 L throughput) by reversed flow of water at 9.6 cm/min. The single vessel for filtration and rupturing of algae was regenerated by passing ozone through the bed for 2 min at 2 L/min when filtration velocity dropped by 78%. However, when ozonation for 2 min did not fully restore the filtration velocity, prolonged ozonation of 5 min was used that confirmed full restoration of filtration velocity.

3.3 Analyses

Chlorococcum aquaticum was cultivated in modified Bristol medium (UTEX) and free of unknown compounds. The only organic matter in the algal sample was presumably the algae. Solids analysis was used to determine the content of algae. Gravimetric solids analyses including total suspended solids (TSS), volatile suspended solids (VSS), total solids (TS), volatile total solids (VTS), volatile dissolved solids (VDS) were measured to determine algal contents (APHA, 2005). The differences in solids after various operations such as filtration, ozonation, and solvent extraction were used to determine filtration, rupture, and yield efficiencies for the individual steps as well as for

the combined operation in the single vessel. Chemical oxygen demand (COD; HACH), soluble chemical oxygen demand (sCOD; HACH) before and after algae rupture were used to determine rupture efficiencies under different ozonation conditions. Particle size and zeta potential of various algal suspensions at different pH were measured by means of dynamic light scattering and laser Doppler microelectrophoresis, respectively, with the instrument Zetasizer Nano ZS (Malvern).

The algae-derived contents were analyzed by GC-MS with electron ionization (EI) source (6890/5973N, Agilent), equipped with a HP-5msi column (30 m, 0.25 mm, 0.25μm; Agilent). Analyses were performed in splitless mode with an injection temperature of 250 ^{o}C, MS detector temperature of 280 ^{o}C, along with the oven temperature program: 50 ^{o}C for 1 min, increasing to 170 ^{o}C at 50 ^{o}C/min, to 300 ^{o}C at 4 ^{o}C/min, and to 320 ^{o}C for 3.6 min at 40 ^{o}C/min. Heneicosanoic acid methyl ester, C21:0, was used as an internal standard at 10 μg/mL. A standard FAME mix (FAME Mix C8-C24, Supelco, PA, USA) was used in conjunction with the internal standard for quantification. For C8:0, C10:0, C12:0, C14:0, C18:0, C20:0, C22:0, and C24:0, the calibration range includes 8, 80, 240, 400, and 800 μg/mL. For C16:1, C18:1, C18:2, and C18:3, the calibration range includes 5, 50, 150, 250, and 500 μg/mL. For C16:0, the calibration range includes 11, 110, 330, 550, and 1100 μg/mL.

CHAPTER 4

RESULTS AND DISCUSSION

The steps of drying the algal mass, obtaining lipid, and converting it into biofuel require significant energy inputs and they can be pivotal to the feasibility of algal lipid as a source of energy. Therefore, I first tested whether the drying step could be eliminated, then tested whether cell rupture, extraction, and transesterification could be performed in a single step, and determined the composition of the resulting biodiesel products (Figure 3.2).

4.1 Sand filtration of algae enabled by agglomeration through charge neutralization

The filtration and backwashing efficiencies of algae after being subjected to different pretreatments that included ozonation and pH adjustment were explored and the results are shown in Table 4.1. Various pretreatments were attempted in order to identify a simple step to promote algal agglomeration for effective sand filtration. Algae concentrations (expressed by different solids concentrations) could vary significantly at different sampling times over the test periods (e.g., different cultivation and stress periods) and influent concentrations into the sand filter could vary according to different pretreatments (e.g., changed dissolved solids after pH adjustments); thus, the

Table 4.1 Effluent solids, filtration efficiencies, and recoveries of pretreated algae through sand filter

	No pretreatment			Ozonation (150 mg O_3/g TSS)			Ozonation, then adjusted to pH 11.7 (150 mg O_3/g TSS, then by 2 M NaOH)			Ozonation, then adjusted to pH 3.6 (by 2 M H_2SO_4)			pH adjusted to 3.3 (by 2 M H_2SO_4)		
	C_0	C_i	C_e	C_0	C_i	C_e	C_0	C_i	C_e	C_0	C_i	C_e	C_0	C_i	C_e
TSS (mg/L)	304	304	170	242	206	80	184	146	52	188	172	20	173	188	11
VSS (mg/L)	284	284	94	216	190	38	180	124	27	178	165	15	168	177	8
TS (mg/L)	838	838	712	758	792	340	784	1782	960	746	820	380	728	874	444
VTS (mg/L)	344	344	148	308	296	152	340	482	198	326	300	98	286	322	78
	SF		R	SF		R	SF		R	SF		R	SF		R
VSS	67		17	82		39	85		20	92		38	95		45

Conditions: Sand size, ≤53 μm (US Sieve No. 270); depth, 21 cm; area, 270 cm^2; bed volume, 5700 cm^3; filtration velocity, 1.5-3.2 cm/ min; gradual decrease of hydraulic head from 12 cm above bed to 0.

Efficiencies calculated by:

$$\text{SF (Sand filtration efficiency)} = \frac{C_0 - C_e}{C_0} \times 100\%$$

$$\text{R (Recovery efficiency)} = \frac{C_b \times V_b}{C_0 \times V_0} \times 100\%$$

where C_0 = Original algae concentration (mg/L), C_i = Influent algae concentration (mg/L), C_e = Effluent algae concentration (mg/L), C_b = Backwashed algae concentration (mg/L), V_i = Influent algae volume = 4 L (= 0.7 bed volume), V_b = Backwash volume = 1.5 L.

concentrations were measured just before filtration of each pretreated samples to establish the initial concentrations, as shown in Table 4.1. Among the solids measurements, VSS was most representative of algal biomass and least perturbed by pretreatments; thus, VSS was selected as a major parameter for calculations of sand filtration (SF) and recovery (R) efficiencies in Table 4.1. Without any pretreatment, 67% of the algal biomass was retained by the sand bed and 17% recovered from the bed by backwashing. Other tested pretreatments were ozonation, ozonation followed by pH adjustment to 11.7, and ozonation followed by acidification to pH 3.6; these pretreatments resulted in SF efficiencies of 82, 85, and 92%, respectively, and R efficiencies of 39, 20, and 38%, respectively. However, the most effective SF and R efficiencies, 95 and 45%, respectively, were achieved by pretreatment in which the suspension pH was simply adjusted to 3.3.

I attributed the increased filtration efficiency through acidification to be a result of reduced repulsion among the like-charged unicellular algal cells and their resultantly increased agglomeration, which occurred due to neutralization of negative charges on the membrane surface brought by the decrease in solution pH. The role of surface charges on biomass in affecting process efficiencies had been reported in the literature (Ives, 1959; Neihof and Loeb, 1972; Uduman et al., 2010). Zeta potential (ζ) indicates the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle, thus indicating the charge condition at the surface of the particles. To ascertain the role of surface charges, particle size and zeta potential of algal suspensions at different pH were measured. Table 4.2 shows decreasing zeta potential from -19.2 to -5.57 mV along with increasing particle size from 117.5 to 2780 nm when

Table 4.2 Major particle sizes and associated zeta potential of algal suspension at different pH

pH	Peak 1 (r, nm)	Peak 2 (r, nm)	Zeta Potential (mV)
8.7	117.5	-	-19.2
7.1	198.9	-	-18.5
6.1	211.1	-	-16.9
5.3	1686	53.07	-14.2
4.9	2479	-	-13.3
3.1	2780	136.4	-5.57
2.8	1399	-	-0.096

the pH was adjusted from 8.7 to 3.1. That particle size increased as the surface potential was neutralized by lowered pH corroborated with the increased algal agglomeration brought by acidification, which enabled retention of the enlarged biomass by the sand bed. Furthermore, the particle size distribution of various suspensions from measurements is shown in Figure 4.1. The particle sizes in the original algal culture and in the sand bed effluent were submicron, while the solids in the pH-adjusted algal suspension (pH 3) and

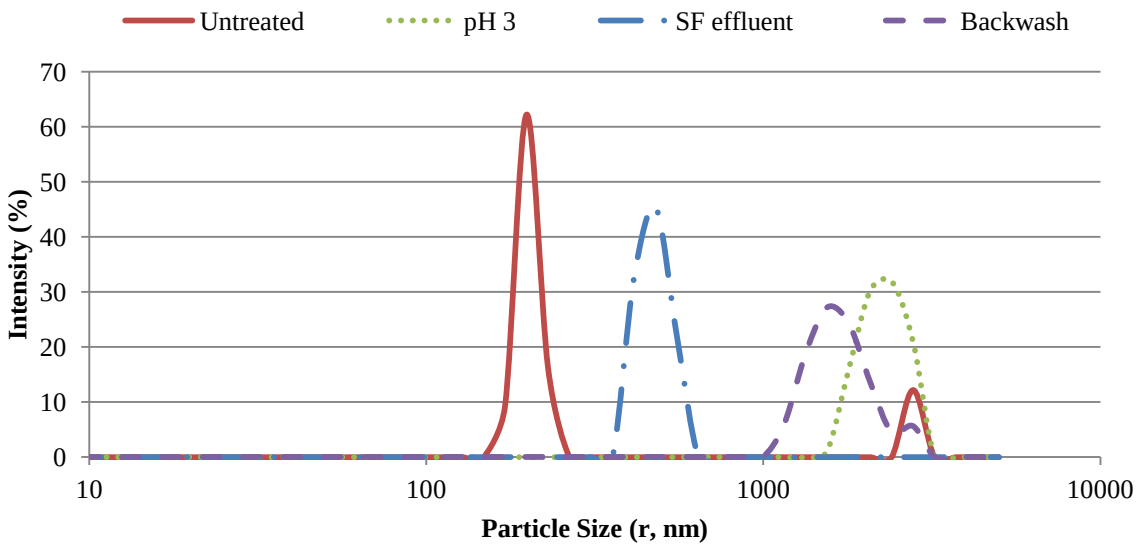


Figure 4.1 Particle size distributions of various algal suspensions, specifically those untreated, acidified, sand-filtered, and backwashed

in the backwash were larger than a micron. This confirmed the increased particle sizes after acidification. In addition, the increase in particle size that resulted in increased removal efficiency might have resulted from straining and sedimentation playing a more significant role in the filtration mechanisms to retain algal biomass in the sand filter. Algal particles larger than the pore space of the sand became strained, agglomerated, and settled in the filter medium (Metcalf and Eddy, 2004), and ultimately formed a cake of filtered algae.

4.2 Rupturing of algae by ozonation

Once the algal biomass is collected in the sand bed, its lipid needs to be obtained for intended utilization. I investigated ozonation as a means to rupture the cell membrane for enhanced lipid recovery. The benefits of delivering ozone via pressure cycles resulting in reduced ozone dosage and increased cell disintegration were extensively discussed in our prior study (Cheng et al., 2012). In a previous study of applying ozone to a batch of activated sludge of 8200 mg/L in tCOD at a dose of 10 mg O_3/g TSS via 20 pressure cycles over 16 min, we found 37-fold increase of the sCOD/tCOD ratio (due to increased soluble COD, i.e., sCOD) and a 25% reduction of TSS, in comparison to a dose of 0.08 g O_3/g TSS via conventional bubbling contact over 15 min that resulted in a 15-fold increase of the sCOD/tCOD ratio and a 12% reduction of TSS. In the present study, 50 L of a cultured algae suspension was passed through the sand filter and most of the biomass retained there; the biomass was backwashed with 1.5 L of water, thus concentrating the algae suspension to a TSS of 2690 mg/L of which the VSS was 1620 mg/L with the latter representing primarily the algal biomass. The concentrated

suspension was then subjected to 20 pressure cycles of ozonation up to pressure of 150 psi, expending 45 mg O_3/g TSS in 21 min. Table 4.3 shows changes of various solids after ozonation. A significant decrease of VSS by 87% from 1620 to 210 mg/L occurred with concomitant increases of VDS by 350% from 42 to 190 mg/L and sCOD by 400% from 45 to 228 mg/L. These indicated solubilization of cell materials including lipid when the membrane enclosure of algae was disrupted by ozone; these results were corroborated by solubilization of COD (increased sCOD/tCOD ratio) from activated sludge disrupted by ozonation (Weemaes et al., 2000; Yeom et al., 2002; Yasui et al., 2005; Bougrier et al., 2007; Dogruel et al., 2007) particularly by ozonation via pressure cycles (Cheng et al., 2012) for the purpose of enhancing solids reduction and energy recovery in subsequent anaerobic treatment. Generally, 50 mg O_3 /g dry solids provides

Table 4.3 Solids changes in algal suspensions after ozonation

Sample	Original conc.	After ozonation by 20 cycles	Rupture Efficiency (RE; %)
TSS (mg/L)	2690	518	80.7
VSS (mg/L)	1620	210	87.0
TS (mg/L)	3190	1000	68.6
VTS (mg/L)	1662	400	75.9
Volatile soluble solid (mg/L)	42	190	-
SCOD (mg/L)	45	228	-

Conditions: 50-L suspension filtered and concentrated into 1.5-L water by backwashing; 20 cycles of ozonation over 21 min (dose of 45 mg O_3/g TSS algae); RE (%) $= \frac{C_0 - C}{C_0} \times 100$ where C_0 and C are algal concentrations before and after ozonation, respectively (mg/L).

adequate treatment (Park and Clark, 2002; Zhang et al., 2009). Cheng et al. found enhanced solubilization even at reduced ozone dosage, and attributed the solubilization of sludge to disintegration of the floc and cell wall that led to release of cell contents to the bulk liquid phase (Cheng et al., 2012).Others found attack of the algal cell enclosure by ozone, which disrupts the cell membrane causing the release of intracellular cytoplasm, microcystins, and volatile organic compounds with increased dissolved organic carbon (DOC) in the aqueous phase (Huang et al., 2008; Miao et al., 2009).

4.3 Extracted compounds by different solvents from wet and dry algal masses

After 24-h Soxhlet extraction of dry algae and wet algae (the latter simply drained of free water), lipid yields using different solvents and their final transesterified FAME products were determined, as shown in Table 4.4.

The results suggested that while n-hexane had been well recognized as an effective solvent for lipid, it was not effective for lipid extraction even after 24 h of Soxhlet extraction of the biomass. Only 0.5 mg of lipid were obtained from the wet biomass amounting to a lipid content of 0.47% in the algae, and from the dry biomass only 1.2 mg were obtained amounting to a lipid content of 1.1%. The extractable lipid contents based on n-hexane were much lower than those based on methanol/chloroform (2:1 v/v) mixture or methanol only. The methanol/chloroform and methanol extracts amounted to 11% and 13% lipid, respectively, from the wet algae, and amounted to 6.8% and 8.1%, respectively, from the dry algae. These results suggested that methanol extracted slightly more lipid than methanol/chloroform did, yet both were much more

Table 4.4 Lipid, esterification product, and FAME amounts obtained after 24-h Soxhlet extraction using different solvents on wet and dry algae

Extraction mode		Lipid[a] (mg)	Lipid content[b] (%)	Esterified product[c] (mg)	Esterified product yield[d] (%)	Esterification efficiency[e] (%)	Identified/quantified FAME[f] (µg)							FAME content[h] (%)
							C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Total[g]	
Wet algae	Hexane	0.50±0.25	0.47±0.22	0.13±0.06	0.13±0.06	16±14	28±15	ND	ND	ND	ND	ND	28±15	24±17
	CH_3OH / $CHCl_3$ (2:1)	11±4.0	11±2.9	4.2±1.3	3.9±0.85	42±6.3	530±70	110±23	21±5.0	24±41	440±93	760±79	1900±300	46±6.0
	CH_3OH	13±3.3	13±1.6	4.6±2.2	4.4±2.3	37±17	610±160	120±39	20±3.0	770±190	470±160	770±190	2000±590	51±24
Dry algae	Hexane	1.2±0.73	1.1±0.59	0.63±0.06	0.60±0.08	73±23	44±10	ND	ND	12±7.0	ND	ND	57 ±15	9.0±3.0
	CH_3OH / $CHCl_3$ (2:1)	7.2±2.0	6.8±0.99	3.5±2.4	3.4±2.5	52±35	290±150	44±21	19±5.0	30±12	90±68	150±78	620±210	21±8.0
	CH_3OH	8.4±2.0	8.1±1.5	2.8±0.58	2.6±0.54	34±4.6	340±110	65±29	17±3.0	35±9.0	220±92	360±150	1000±400	36±8.0

Values are mean ± standard deviation of triplicates; ND = not detected.

[a] Residual amount after filtration (1.2 µm) and solvent evaporation.

[b] Lipid content (%) = Lipid collected (mg)/ Algal mass (mg; dry basis) x 100%. Dry mass of wet sample was estimated based on equal sample amount (i.e., 104.7±19.13 mg/L).

[c] Esterification at 80 ^oC for 30 min after addition of CH_3OH (3.4 mL) and H_2SO_4 (0.6 mL); amounts obtained after extraction, filtration (1.2 µm), and solvent evaporation.

[d] Esterified product yield (%) = Esterified product (mg)/ Algal mass (mg; dry basis) x 100%. Equal dry algal mass assumed for wet samples (i.e., 104.7±19.13 mg/L)

[e] Esterification efficiency (%) = Esterified product (mg)/ Lipid collected (mg) x 100%.

[f] Identified/quantified FAMEs were by GC/MS on the esterified product.

[g] Total FAME (mg) = Sum of C16:0, C16:1, C18:0, C18:1, C18:2, and C18:3

[h] Total FAME Content (%) = Total FAME (mg)/ esterified product (mg) x 100%.

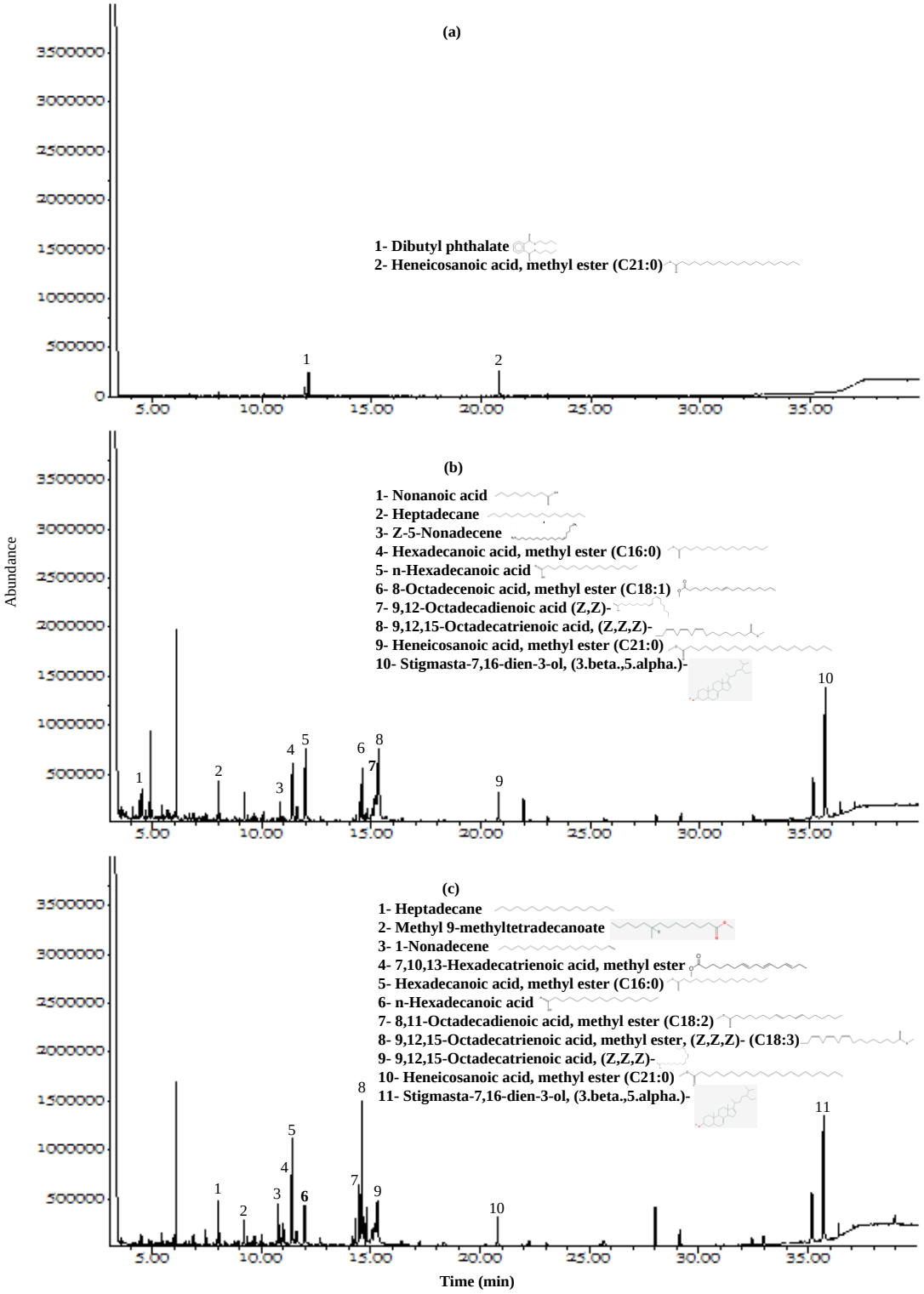
Other conditions: Initial algal concentration as SS_0 = 120±16 mg/L, VSS_0 = 110±11 mg/L; algal mass procured by filtration through 1.6-µm filter; water content in algae was 75% before drying; extraction solvent volume = 210 mL with n-hexane, methanol/chloroform (2:1 v/v), and methanol; esterification.

effective than n-hexane as a solvent.

For comparison of dry and wet algal samples, neither had been mechanically ground to destruct the membrane; n-hexane extracted poorly. Hexane was likely prevented from intimate contact with the cell membrane because of a substantial water layer coating protecting the membrane particularly with the wet algae. The proteins and polysaccharides at the cell exterior, which are not miscible with n-hexane, might also have protected it from the solvent. On the other hand, methanol being a polar solvent was effective in extracting lipid from the algae even without drying and grinding the biomass, demonstrating the solvent's ability to interact with the surficial water layer and cell wall components to rupture the cell during extraction. Since methanol was an effective solvent, being environmentally benign and a reagent in transesterification, it was used in the rupture and transesterification step.

Extracted compounds from wet and dry algae using different solvents were analyzed by GC-MS, and the results are shown in Figure 4.2. A qualitative examination of the chromatograms confirmed that n-hexane extracted few compounds from the wet or dry biomass, albeit slightly more from the dry (Figure 4.2a & 4.2d). Among the three solvents (n-hexane, methanol/chloroform (2:1 v/v), and methanol), methanol extracted the most compounds (Figure 4.2c & 4.2f). The relative abundance signals from the wet vs. the dry algae (left vs. right chromatograms) appeared to be comparable, except that 9,12-octadecadienoic acid and 9,12,15-octadecatrienoic acid were more efficiently extracted from the wet samples. Further quantification of the final products from different routes are discussed below. Note that while the lipid was expected as product, the identified compounds were transesterified products that indicated released ester compounds from

Figure 4.2 Products and relative amounts obtained after 24 h of Soxhlet extraction in different modes (a) wet algae with n-hexane, (b) wet algae with methanol/chloroform (2:1 v/v), and (c) wet algae with methanol (d) dry algae with n-hexane, (e) dry algae with methanol/chloroform (2:1 v/v), and (f) dry algae with methanol


(a)
1- Dibutyl phthalate
2- Heneicosanoic acid, methyl ester (C21:0)
(b)
1- Nonanoic acid
2- Heptadecane
3- Z-5-Nonadecene
4- Hexadecanoic acid, methyl ester (C16:0)
5- n-Hexadecanoic acid
6- 8-Octadecenoic acid, methyl ester (C18:1)
7- 9,12-Octadecadienoic acid (Z,Z)-
8- 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
9- Heneicosanoic acid, methyl ester (C21:0)
10- Stigmasta-7,16-dien-3-ol, (3.beta.,5.alpha.)-
(c)
1- Heptadecane
2- Methyl 9-methyltetradecanoate
3- 1-Nonadecene
4- 7,10,13-Hexadecatrienoic acid, methyl ester
5- Hexadecanoic acid, methyl ester (C16:0)
6- n-Hexadecanoic acid
7- 8,11-Octadecadienoic acid, methyl ester (C18:2)
8- 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (C18:3)
9- 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
10- Heneicosanoic acid, methyl ester (C21:0)
11- Stigmasta-7,16-dien-3-ol, (3.beta.,5.alpha.)-
Abundance
Time (min)

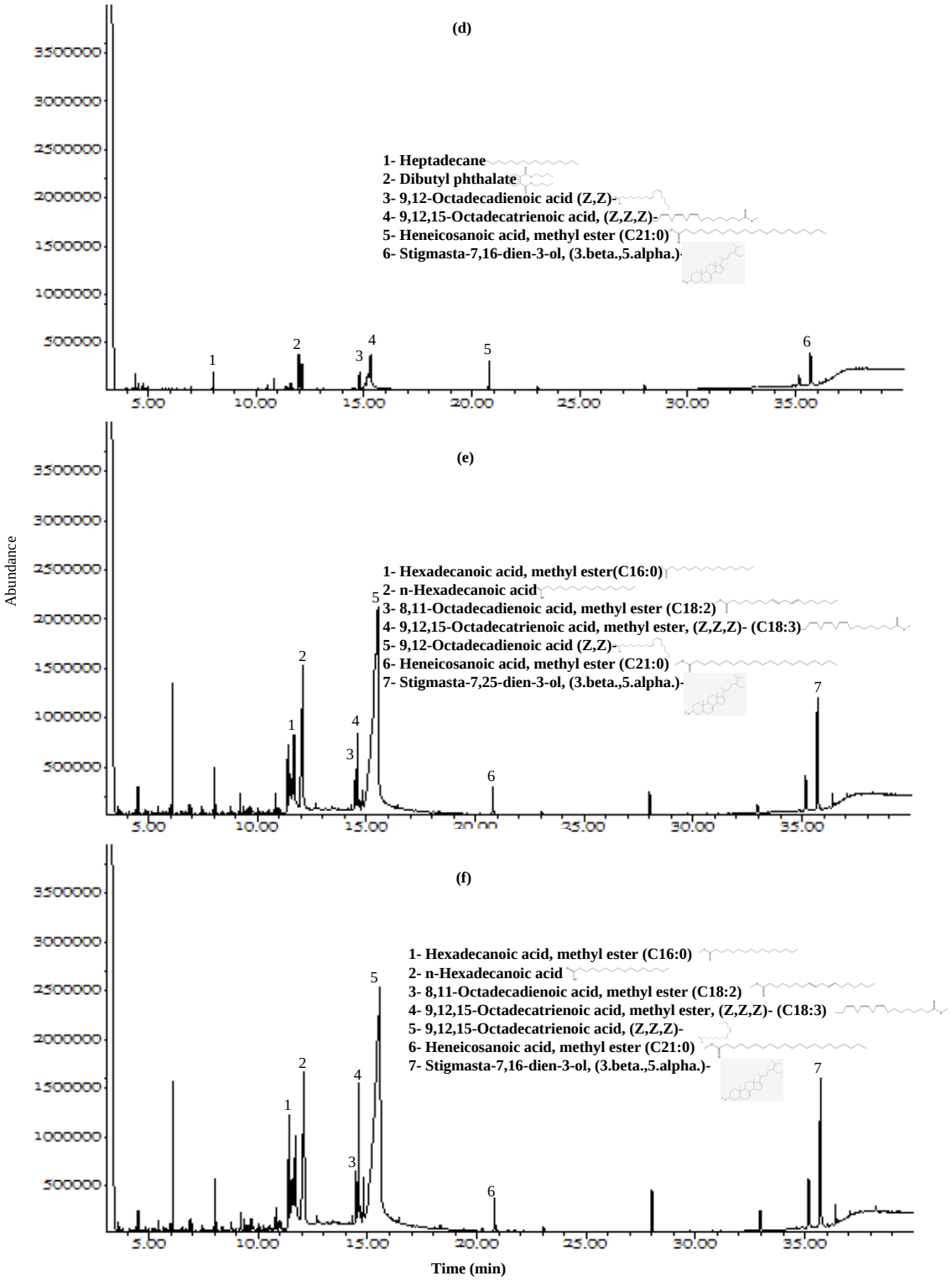


Figure 4.2 Continued

the dissolved membrane and/or partial transesterification of released lipid with methanol during extraction at the reflux temperature of 65 ^{o}C.

Table 4.4 also shows FAME contents along with structural information on the numbers of carbons and unsaturated bonds (e.g., C16:1 indicating a FAME compound of 16 carbons with 1 double bond) when lipid samples from different routes (e.g., solvents, wet or dry) were transesterified. Only trace amounts of FAMEs resulted from either wet (i.e., 28 µg as C16:0) or dry (57 µg of mostly C16:0 and some C18:1) samples when n-hexane was used for extraction, due to the solvent's poor extraction ability. Based on FAME yields, methanol was more desirable as an extraction solvent because it required no drying before transesterification; the yield of 51% with methanol without prior drying was the highest, totaling 2000 µg of FAME constituted mainly by 16-18 carbons with 0-3 unsaturated bonds from approximately 100 mg of algal mass (dry basis) that accounted for a FAME yield of 2% derived from the algal biomass. Both the lipid yield and transesterified product yield (13% and 4.4%, respectively) from the wet algae were significantly greater than the corresponding yields (8.1% and 2.6%, respectively) from the dry algae, which suggested that the extraction of lipid and transesterification from the wet sample in a single step suffered no prohibitive effect. The increased transesterified product might have been due to residual water carried from the wet sample; however, the yield comparison of total FAME based on quantification by GC-MS precluding residual water still indicated higher FAME content from the wet sample (51%) than from the dry sample (36%). Based on the collected transesterified product, the yield was highest at 4.4% from the wet algal mass, and the transesterification efficiency from lipid was 37%. Johnson and Wen (2009) found that both biodiesel yield and FAME content from direct

transesterification of wet biomass obtained by centrifugation were significantly lower than from dry biomass obtained by freeze-drying. The efficiencies improved when they performed extraction and transesterification separately in consecutive steps. In this study, while the transesterification efficiencies appeared to be less overall (all solvents considered) for the "wet lipid," the overall product yield and FAME content in the transesterified product were higher with the wet algae. Thus, the extraction and transesterification of wet algal mass without drying were viable with the hydrophilic solvent methanol.

4.4 Ozonation of algae and direct transesterification conversion

Chemically rupturing the algal membrane was thought to be useful in accelerating lipid release when the solvent was not afforded prolonged contact with the biomass. I introduced the wet algae into CH_3OH and subjected the suspension to ozonation to determine if it would enable lipid release quickly and completely, which might lead to increased lipid yield and subsequent FAME formation. Positive outcomes from such would obviate the energy-intensive, commonly practiced steps such as drying, grinding, and solvent extraction prior to transesterification. Table 4.5 shows significant effects of ozonation on the acquired lipid amounts, transesterified products, and FAME yields. The lipid yield increased with increasing contact time, from 8.3% without ozonation to 10%, 12%, 13%, and 15% corresponding to 1, 3, 5, and 10 min of ozonation. Note that even in the case without ozonation, a significant amount of lipid was collected after the algae had been in contact with methanol for 1 h; this duration was replicated for all samples for consistency when various degrees of ozonation were applied, and it allowed methanol to

Table 4.5 Lipid, esterification product, and FAME amounts obtained from algae subjected to various degrees of ozonation

Ozonation time (min)	Lipid collected[a] (mg) & Lipid content[b] (%)	Transesterified product[c] (mg) & Transesterified product yield[d] (%)	Transesterification efficiency[e] (%)	Identified/quantified FAME[f] (μg) in lipid before transesterification & Identified/quantified FAME[f] (μg) after lipid transesterification							Total FAME Content[g] (%) in lipid collected & Total FAME Content[g] (%) in transesterified product
				C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Total[7]	
0	8.7±4.2 8.3±2.6	1.5±0.36 1.5±0.27	15±6.7	92±43 470±290	19±1.7 67±39	5.0±8.6 23±9.0	19±3.2 31±23	210±64 380±220	320±82 680±430	670±200 1600±980	7.5±5.0 & 100±45
1	11±3.3 10±1.8	1.8±0.95 1.7±0.39	14±3.1	58±22 610±370	5.2±9.0 67±46	8.0±6.9 31±13	13±8.3 32±9.0	85±84 430±230	110±130 760±560	270±250 1900±1200	2.0±1.3 & 100±29
3	13±3.9 12±1.4	1.3±0.85 1.2±0.44	9.1±2.8	55±15 650±290	4.5±7.7 32±33	8.9±7.7 31±8.0	2.9±2.8 51±6.0	18±30 150±200	19±32 220±380	110±91 1100±900	0.70±0.35 & 80±14
5	14±3.9 13±1.4	1.1±1.1 0.91±0.66	8.2±4.8	67±27 610±160	ND ND	12±0.86 31±7.0	ND 7.0±7.0	8.6±12 43±39	ND 59±69	85±25 710±270	0.63±0.27 & 98±50
10	16±4.3 15±4.0	1.1±0.70 1.0±0.35	6.0±2.9	84±35 630±280	ND ND	13±0.74 31±9.0	ND 5.6±9.8	ND ND	ND ND	97±35 660±300	0.58±0.28 & 65±12

Wet algae obtained by filtration (1.6 μm); same footnotes as Table 4.4.

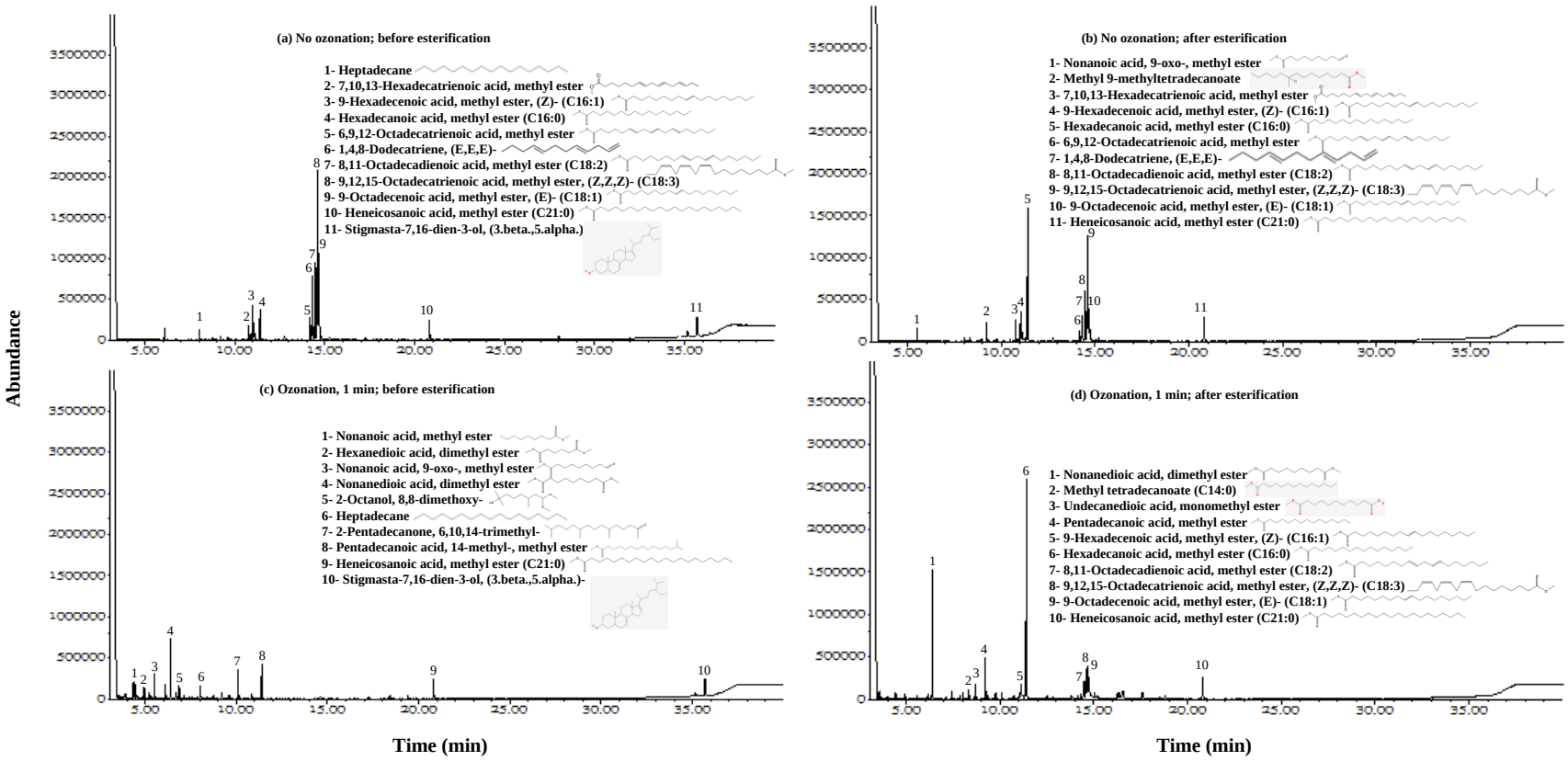
compromise the algal cell to some degree resulting in lipid release to form the solution. However, the transesterified product yield increased from 1.5% to 1.7% after 1 min of ozonation; further ozonation resulted in gradual decreases to 1.0% after 10 min of ozonation. The transesterification efficiency was adversely impacted when longer ozonation time (e.g., > 1 min) was used, from 15% without ozonation to 14% at 1 min of ozonation and to 6.0% at 10 min of ozonation. This was attributed to destruction of the transesterified compounds upon prolonged contact with ozone. While ozonation increased the collected amount of lipid within minutes, it acted to decrease the transesterified products beyond a brief exposure.

Ozonation impacted the composition of the FAME mixture and its constituent percentage in the whole transesterified product. Without ozonation or with only brief ozonation (1 min), identified FAME compounds accounted for 100% of the transesterified product. With longer periods, e.g., 3 min, FAME composition shifted with a decrease of C18 from 1300 to 450 µg, while the presence of quantifiable FAME dropped from 100% to 80% in the total transesterified product. With 10 min of ozonation, unsaturated FAME disappeared almost completely, leaving primarily smaller, fully saturated C16:0 and C18:0 that accounted for 95% and 5%, respectively, of the total FAME. Ozone caused fragmentation of the unsaturated C18 into smaller saturated C16 and likely into other smaller compounds at low concentrations. The results show that brief ozonation increased transesterified product yield slightly (by 1.7%). Besides, comparing with FAME content in Table 4.4, this method provided a higher FAME content (65-100%) than Sohxlet extraction (9-51%) which means that the ozone would remove those other than FAME impurities in final product. However, an optimal contact

time should be determined more accurately as a long exposure was counterproductive for yield and efficiency.

Figure 4.3 identifies the compounds before and after transesterification and the effect of increasing ozonation on product distribution; these compounds have already been quantified in Table 4.5. Even without deliberate transesterification, identifiable products consisted mainly of transesterified FAMEs, which might have resulted from: 1) dissolution of the phospholipid membrane by CH_3OH thus releasing the ester constituents, and 2) partial transesterification of the released lipid when CH_3OH compromised the cell membrane during contact. The compounds were mainly C16 – C18 with unsaturated bonds that accounted for the "lipid" amount collected without ozonation (time zero) of Table 4.5, albeit significantly less than the FAME products after transesterification. The smaller amount of collected lipid without ozonation (8.3% of algal mass) was due to partial release of lipid, which was then converted into FAMEs of Figure 4.3a in CH_3OH. The quantification results of Table 4.5 indicated that prior to transesterification FAMEs accounted for 7.5% of this "lipid" portion (heavy triacylglyceride (TAG) compounds were not observed by GC) and that after transesterification FAMEs accounted for 100% of the entire transesterified product. Table 4.5 also shows consistently higher FAMEs after transesterification at different ozonation durations. Furthermore, dimethyl esters and trimethyl esters were formed with ozonation. Ozone attack of the hydrocarbons resulted in formation of carboxylic group(s) on the molecule, which then reacted with CH_3OH to become esters. Product composition shift (as discussed for Table 4.5) aside, prolonged contact of the lipid with ozone for 10 min produced smaller fatty acid molecules such as heptanoic and nonanoic acids, apparently from fragmentation at the double bond of the

Figure 4.3 GC/MS identification of products after cell rupture and subsequent conversion after various degrees of ozonation (a) no ozonation; before esterification, (b) no ozonation; after esterification, (c) ozonation 1 min; before esterification, and (d) ozonation 1 min; after esterification (e) ozonation 3 min; before esterification, (f) ozonation 3 min; after esterification, (g) ozonation 10 min; before esterification, and (h) ozonation 10; after


(a) No ozonation; before esterification
1- Heptadecane
2- 7,10,13-Hexadecatrienoic acid, methyl ester
3- 9-Hexadecenoic acid, methyl ester, (Z)- (C16:1)
4- Hexadecanoic acid, methyl ester (C16:0)
5- 6,9,12-Octadecatrienoic acid, methyl ester
6- 1,4,8-Dodecatriene, (E,E,E)-
7- 8,11-Octadecadienoic acid, methyl ester (C18:2)
8- 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (C18:3)
9- 9-Octadecenoic acid, methyl ester, (E)- (C18:1)
10- Heneicosanoic acid, methyl ester (C21:0)
11- Stigmasta-7,16-dien-3-ol, (3.beta.,5.alpha.)
(b) No ozonation; after esterification
1- Nonanoic acid, 9-oxo-, methyl ester
2- Methyl 9-methyltetradecanoate
3- 7,10,13-Hexadecatrienoic acid, methyl ester
4- 9-Hexadecenoic acid, methyl ester, (Z)- (C16:1)
5- Hexadecanoic acid, methyl ester (C16:0)
6- 6,9,12-Octadecatrienoic acid, methyl ester
7- 1,4,8-Dodecatriene, (E,E,E)-
8- 8,11-Octadecadienoic acid, methyl ester (C18:2)
9- 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (C18:3)
10- 9-Octadecenoic acid, methyl ester, (E)- (C18:1)
11- Heneicosanoic acid, methyl ester (C21:0)
(c) Ozonation, 1 min; before esterification
1- Nonanoic acid, methyl ester
2- Hexanedioic acid, dimethyl ester
3- Nonanoic acid, 9-oxo-, methyl ester
4- Nonanedioic acid, dimethyl ester
5- 2-Octanol, 8,8-dimethoxy-
6- Heptadecane
7- 2-Pentadecanone, 6,10,14-trimethyl-
8- Pentadecanoic acid, 14-methyl-, methyl ester
9- Heneicosanoic acid, methyl ester (C21:0)
10- Stigmasta-7,16-dien-3-ol, (3.beta.,5.alpha.)-
(d) Ozonation, 1 min; after esterification
1- Nonanedioic acid, dimethyl ester
2- Methyl tetradecanoate (C14:0)
3- Undecanedioic acid, monomethyl ester
4- Pentadecanoic acid, methyl ester
5- 9-Hexadecenoic acid, methyl ester, (Z)- (C16:1)
6- Hexadecanoic acid, methyl ester (C16:0)
7- 8,11-Octadecadienoic acid, methyl ester (C18:2)
8- 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (C18:3)
9- 9-Octadecenoic acid, methyl ester, (E)- (C18:1)
10- Heneicosanoic acid, methyl ester (C21:0)
Abundance
Time (min)
Time (min)

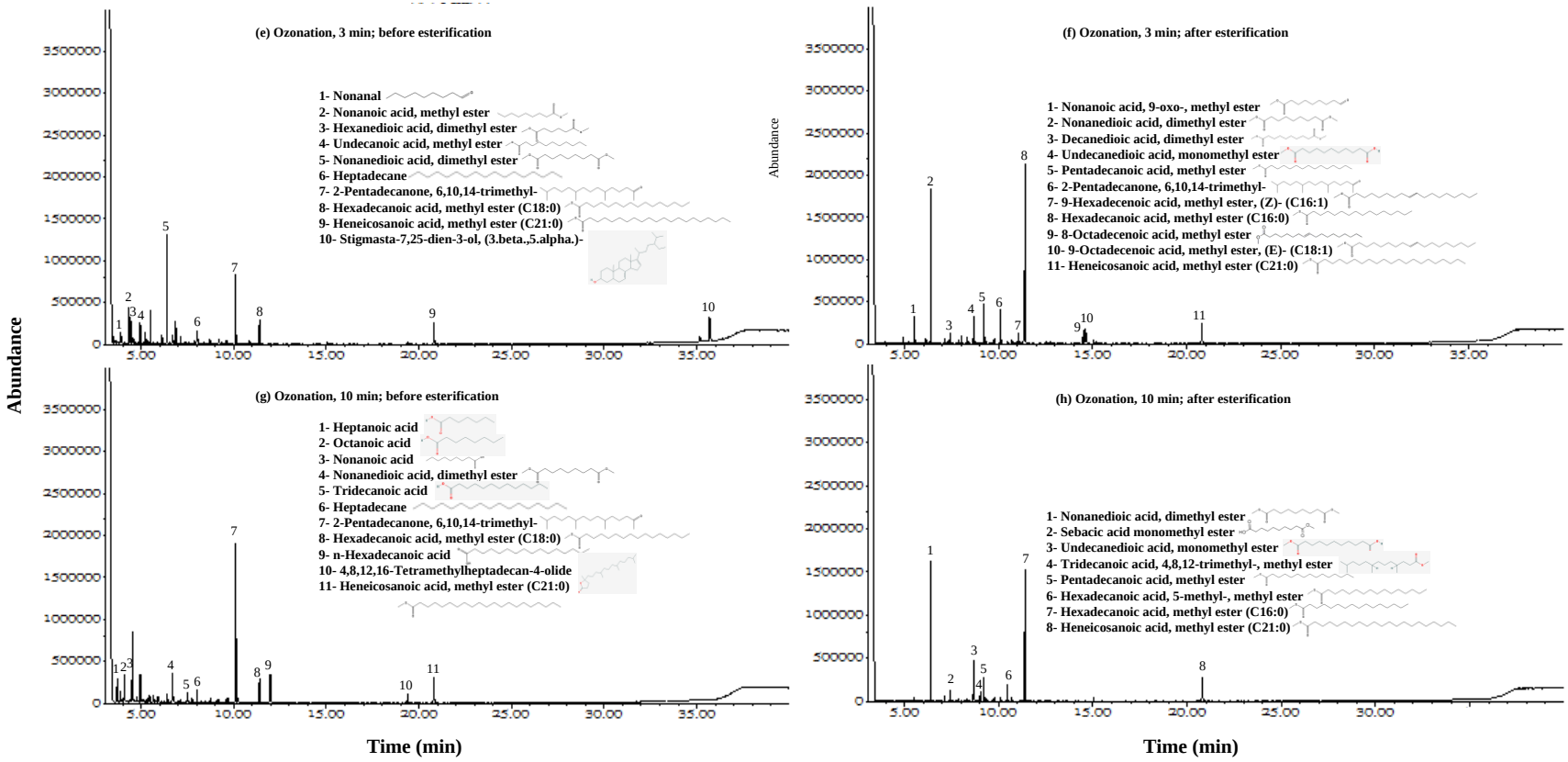


Figure 4.3 Continued

parent C16-18 compounds, or elimination of CO_2 at terminal ends. These smaller fatty acids also resulted in smaller FAMEs upon esterification.

The composition trend of FAMEs from ozonated biomass was consistent with a previous study that observed increased saturated compounds with increased ozonation (Huang et al., 2014). Thus, ozonation brought forth a potential tool to alter the kinds and compositions of the FAME products. This tool may provide a balance between the oxidative stability offered by the saturated hydrocarbons and the lower melting points offered by the unsaturated hydrocarbons for cold climate applications. For example, smaller, saturated FAME could be favorably produced by pretreatment of the algae with ozone, while larger, unsaturated FAME with lower melting points could be favorably produced without ozonation. Optimizing the ozone dose could be used to favor desirable product composition.

Figure 4.4 shows algae that were collected by filtration, placed in test tubes, added with CH_3OH, inverted several times, and then subjected to bubbling ozone stream for various durations of 0, 1, 3, 5, and 10 min, from right to left test tubes, respectively. The algal mixture was green before ozonation (rightmost) and became increasingly pale with increasing ozonation (right to left). The disappearance of the green color with ozonation corroborated the rupture of protective cell membrane and chloroplast enclosure, resulting in exposure of the chlorophyll to ozone that led to its destruction and color disappearance. Ozonation provides means of disrupting cell membrane by either direct attack by the molecular ozone and attack by secondary oxidant OH radical created by decomposition of ozone in water; both forms of attack on the cell wall disrupt the cell's structural integrity, enabling the organic solvent to cell contents including the fatty

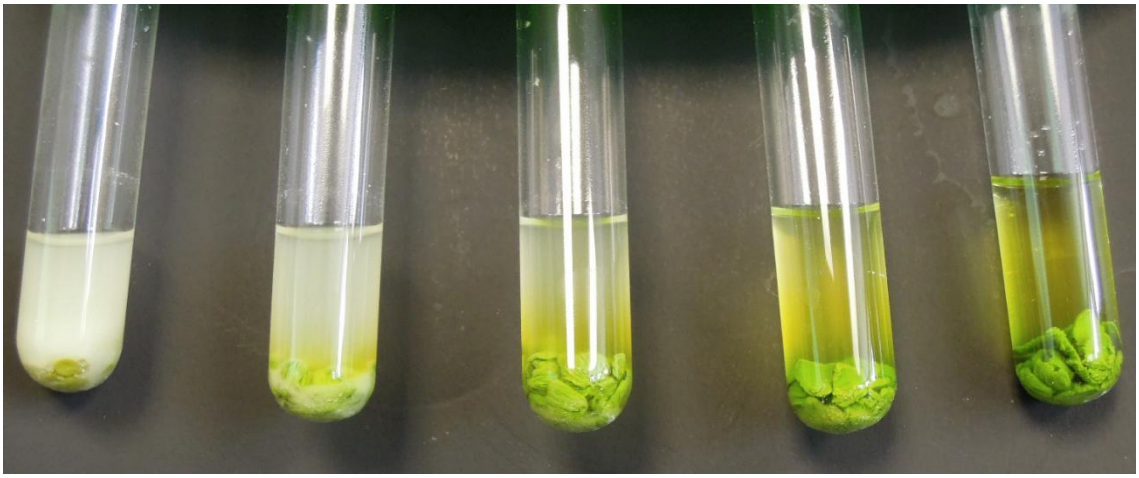


Figure 4.4 Ozonation of collected algae for varying durations, 0, 1, 3, 5, and 10 min, from right to left

acids and further oxidation and fragmentation of cellular substances into smaller molecules. The fatty acids and hydrocarbons in samples after ozonation in organic solvent were heptadecene, hexadecenoate, octenal, nonanol, and others, similar to those found in aqueous phase as previously reported (Huang et al., 2008; Miao et al., 2009).

4.5 Product yield and FAME content in streamlined processing

The primary objective of this work was to streamline the processing of cultivated algae into biofuel, without the drying and extraction steps prior to transesterification. The transesterified product yield was highest at 1.7% consisting completely of FAME from briefly ozonated (1 min) algae.

While the product was completely FAME, the yield was low compared to other studies, such as to a yield of 57% of crude biodiesel with a FAME content of 66% (Johnson and Wen, 2009) from *S. limanicum*, albeit their yield was lower when the algae was wet without prior freeze-drying. A direct comparison of process yield was not warranted because of differences in the species, sample moisture, and particularly

cultivation conditions and growth stage that this study did not attempt to control. Note that in this study the only criterion for harvesting the algae for experiments was when the cultivation batch reached a suspended solid concentration of 100 mg/L, prior to which the batch was maintained under favorable growth conditions without special periods such as stress to promote lipid production. Even within this study, the results of Tables 4.4 and 4.5. were obtained from two batches likely differing in their growth stages. The results of Table 4.4 suggested that the transesterified product yield and FAME content were higher from the wet sample than from the dry, based on CH_3OH as the solvent carrier. The results of Table 4.5 showed brief ozonation (e.g., 1 min) with higher product yield, consisting of only FAME, from the wet and ozonated sample. Thus, the results demonstrated that the streamlined scheme by ozonation of wet algae in CH_3OH and immediately continued by transesterification of the dissolved lipid was viable, eliminating the need to dry and extract prior to transesterification. This assessment was based on comparisons of yield and composition of the wet, ozonated route with other routes involving drying and extraction in this study. The overall low yield of the transesterified product may be related to transesterification efficiency that was not optimized; it calls for further optimization of the conversion process.

4.6 Algae collection, cell rupture, and lipid extraction in one operation vessel and bed regeneration

Figure 4.5 shows algal throughput (initial VSS of 73 mg/L) vs. time profiles during sand filtration operation. For a newly packed sand bed, filtration velocity for distilled water (DW) was steady at 1.2 cm/min (Line D0). After three consecutive runs,

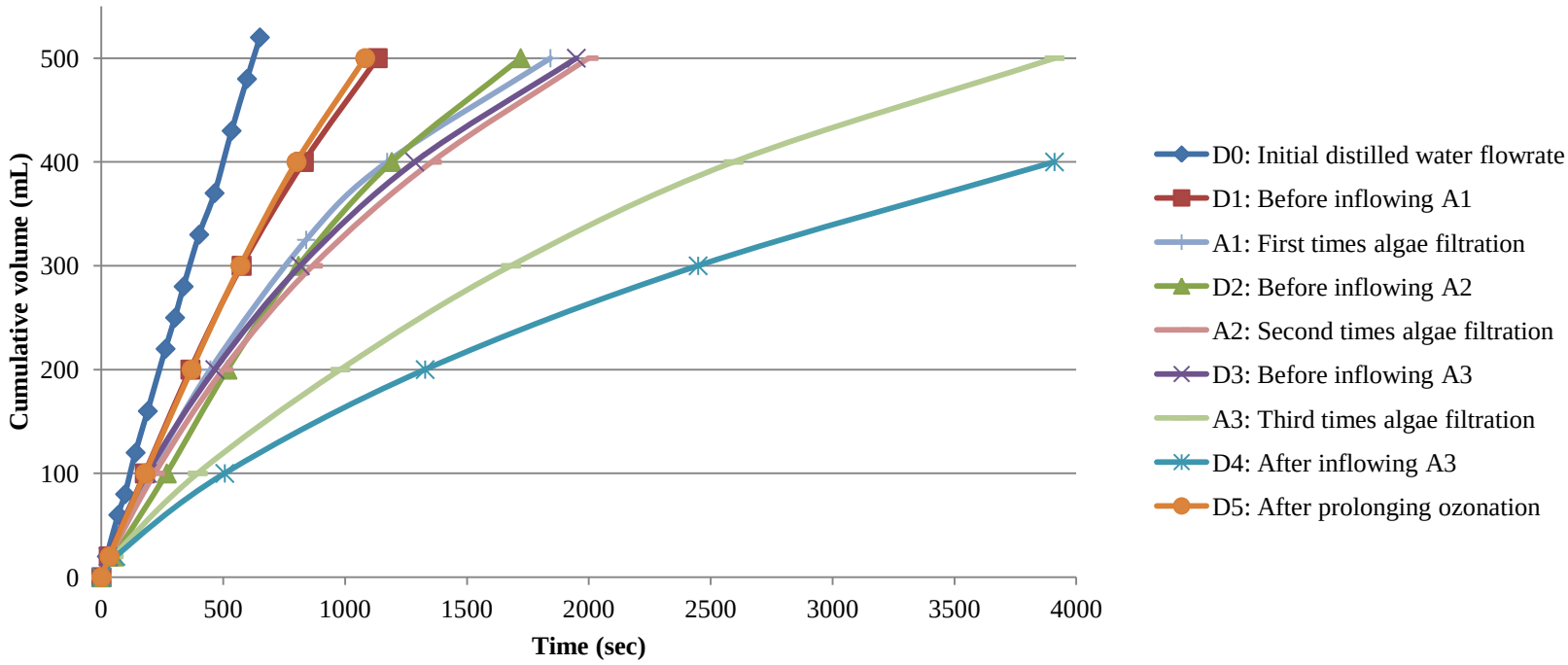


Figure 4.5 Volume vs. time profiles of algal suspension (AW) and distilled water (DW) during consecutive filtration runs; the order of throughputs and operations were: DW of new sand bed (Line D0), DW through a regenerated bed (Line D1), AW (Line A1) and collection, DW (Line D2), AW (Line A2) and collection, DW (Line D3), AW (Line A3) and collection, DW (Line D4), regeneration by ozonation in methanol, DW (Line D5). Conditions: Sand size, ≤53 μm (USA standard testing sieve No. 270); depth, 6.5 cm; area, 40 cm^2; bed volume, 260 cm^3; filtration velocity, 0.15-1.2 cm/ min; constant 7.5 cm above the sand for the first 0.3 L and gradual decrease to 0 in the remaining 0.2 L

filtration velocity can be reestablished at 0.67 cm/min (Line D1). The first algal suspension (0.5 L VSS of 64 mg/L) was passed through the bed under the influence of hydraulic head (constant 7.5 cm above the sand for the first 0.3 L and gradual decrease to 0 in the remaining 0.2 L); while showing an average filtration velocity of 0.41 cm/min in this first run, the velocity profile developed a curvature (Line A1) as it progressively slowed due to the pore space being filled and decreasing hydraulic head near the end. After draining the remaining water, the column was added with 90 mL of methanol reaching a column height of 2 cm above the sand surface; the sand bed was then ozonated for 2 min (through the sand bottom at 2 L/min of 1.5% O_3) and afterward the lipid methanol extract was drained from the column. The regeneration and algal collection via ozonation in methanol returned the filtration velocity of DW to 0.44 cm/min (Line D2), thus completing the first cycle of algae filtration and regenerative lipid collection. The second cycle of filtration and regenerative collection was carried out likewise that resulted in filtration velocity of 0.38 cm/min (Line A2), and the third cycle in much slower filtration velocity of 0.2 cm/min (Line A3). While the second regeneration restored the filtration velocity of DW to 0.39 cm/min (Line D3), the third regeneration restored the filtration velocity of DW to only 0.15 cm/min (Line D4). Thus, following the third lipid collection, the sand bed was subjected to longer ozonation for 5 min, which fully restored the filtration velocity of DW to 0.70 cm/min (Line D5), similar to that at the start of operation (Line D1).

The apparent lipid yield from algae was determined to be 15.7%, based on the total suspended solids (TSS) as the lipid content carried out from the sand bed by the methanol extract following ozonation. This yield is very close to that (15.7% incidentally)

obtained by direct Soxhlet extraction of the filtered, wet biomass with methanol for 24 h. These results showed that ozonation of the algae-loaded sand bed for 5 min was capable of both restoring the filtration velocity to its initial value (0.7 cm/min) and of removing the algal lipid from ruptured algal cells through dissolution in methanol used as an ozonation medium. The filtration and rupture processes are likely to be scaled up on the basis of ozone dose per unit algal mass. It should be noted that rupturing of algae and bed regeneration was accomplished via conventional bubbling of ozone gas, and the employed 2 min for rupturing and 5 min for regeneration did not represent optimal periods. As such, the ozone expenditure was estimated to be 1.8 g O_3/g TSS. The dose (1.8 g O_3/g TSS) is likely when ozonation is optimized or pressure-assisted ozonation is incorporated. Previous rupturing results of activated sludge showed more effective rupturing of biomass by PAO with only one-eighth of the dose by conventional ozonation, as cited in Phase II: Rupturing of algae by ozonation (Cheng et al., 2012).

4.7 Current lipid collection methods and cost

Two costly steps in procuring algal lipid involved harvesting (i.e., concentration of algae) and extraction; Brentner et al. (2011) identified centrifugation for harvesting algae that required 90% of the total energy gained in algal biodiesel production or press filtration that required 79% of the total energy gained from production. Following dewatering, a subsequent step of solvent extraction of algal lipid would require an additional 10% of the total energy gained in biodiesel production, amidst other more energy-intensive alternative routes such as supercritical CO_2 and ultrasonication processes that required additional amounts of 66% and 110%, respectively, of the total

energy gained from biodiesel production. Therefore, dewatering and lipid extraction would have consumed the entire energy budget gain in biodiesel production. Clearly, harvesting and extraction must be made more energy-efficient. The use of sand filtration followed by rupturing of algae with ozone in methanol developed in this study required minimal energy input to the dewatering and lipid extraction processes. Specifically, ozone generation requires electricity of 8-17 kWh/kg O_3 (i.e., 0.8 to \$1.7/kg O_3 based on electricity price of \$0.10/kWh). Assuming an ozone dose of 50 g O_3/kg dry algae, which has been found effective for rupturing algae in this study (Table 4.3) and for rupturing activated sludge by Cheng et al. (2012), electrical energy to generate O_3 for rupturing algae would be 1.4-3.1 MJ /kg dry algae. The energy cost of rupturing algae with O_3 at 2 MJ/kg algae is a fraction of the energy used to produce biodiesel, ~40 MJ/kg biodiesel or ~6 MJ/kg algae (assuming 15% of lipid content can be converted to similar mass of biodiesel), a smaller fraction (2/6 = 33%) than that (>90%) with centrifugation and solvent extraction. The energy estimates here do not account for all of the energy that may be required for a full scale system based on this technique, but it is evident from this work that use of this methodology offers the potential for using less energy than centrifugation and solvent extraction. It should be noted that the estimated dose of ozone (0.05 g/g) without pressure cycles appears to be conservative and must be further optimized in pilot scale.

4.8 Pilot scale design

The consecutive ozonation and esterification of wet algae undertaken would extend acidification and sand filtration to collect the cultivated algae. Thus, I propose a

complete streamlined processing scheme from a cultivated algal suspension to the biodiesel product as illustrated in Figure 4.6a, which bypasses energy-intensive steps such as centrifugation, drying, and solvent extraction. In Figure 4.6b, three stacked sand filtrations can save land usage and still provide three times the surface area for a big treatment amount. To use the scaled-up design with assumptions listed in Table 4.6 and design parameters shown in Table 4.7, an intermediate step of experimentation and verification would be desirable before the final design. With a scaling factor of 600 on the filtration surface area from the laboratory benchtop experiments, it would require three sand filters with supporting collection, rupture, and regeneration, requiring 20 m^2 to process 2200 m^3 of algal suspension and collect 240 kg of VSS as algal biomass per day. After addition of 13 m^3 of recyclable methanol and purging with 420 kg O_3 per day through the sand column, 23 kg of lipid per day can be obtained in dissolved form in methanol along with other biomass residual; the mixture is to be transferred into a separation tank to separate the lipid in methanol and the biomass residual for biogas generation. The lipid in methanol is to be transesterified biodiesel products, 3,270 g of biodiesel or one gallon per day.

Afterward, the loaded sand filter, byproduct glycerol, excess methanol, and wash water should be regenerated, purified, recycled, and reused, respectively. Pending on verification of processing at an intermediate scale, the pilot-scale parameters as listed in Table 4.7 will then be ready to be implemented to verify the feasibility and determine the costs to produce 1040 gallon biodiesel per day. Since the laboratory scale of biodiesel production is very small, it would require a very large scaling factor to obtain an economic estimate for commercial production, which renders the estimate hardly

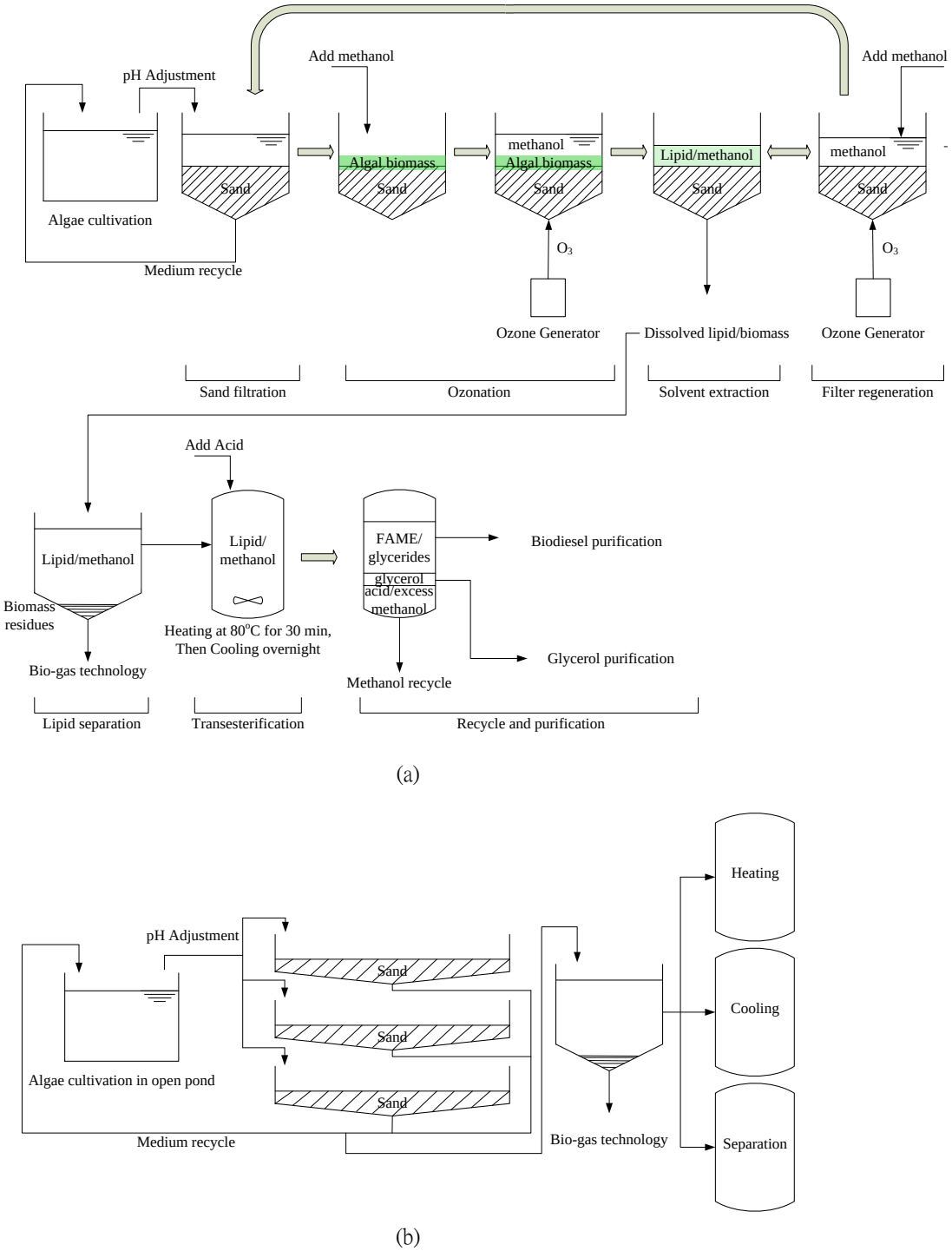


Figure 4.6 Pilot scale process (a) Integral process scheme (b) Stacked sand filtration pilot scale design

Table 4.6 Assumption parameters

Process	Assumption	
Growth	Algae concentration	110 mg/L VSS
	Lipid content	10%
Harvest	pH adjustment H_2SO_4 added	0.0001 % concentrated sulfuric acid
	Sand filtration velocity	3 cm/min
	Collection time	20 hr/day
	Sand filtration efficiency	95%
	Sand filtration operation	
Rupture	Ozone dosage	1.8 g O_3/ g VSS
	Ozone operation	
Conversion	Conversion efficiency	14 %
	Lipid Molecular weight	250-290 (270) g/mol
	MeOH ratio	MeOH to algal lipid molar ratio 6:1
	H_2SO_4 ratio	2% concentrated sulfuric acid in MeOH
	Water for purification of biodiesel	Washing utilizes water equal to 20% w/w of biodiesel feed
	FAME content in final converted product (biodiesel)	100 %
	Density of biodiesel at 15 ^oC	0.85 g/cm^3

Table 4.7 Design parameters of a pilot scale processing system

Process	Design parameters	Intermediate Scale	Pilot Scale
Harvest	pH adjustment H_2SO_4 added	2.8 L /day	3 m^3/ day
	Sand filters	3	3
	Sand filter surface area	20 m^2	22,000 m^2
	Hydraulic high	12 cm	12 cm
	Sand column depth	21 cm	21 cm
	Flow rate	620 L /min /column, 2200 m^3 / day	650 m^3/min/column, 2,300,000 m^3/ day
	Volume for sand filter column	7 m^3	7100 m^3
	Treated algae solution	2200 m^3/ day	2,300,000 m^3/ day
	Collected algae biomass	240 kg VSS / day	240,000 kg VSS/day
Rupture	Ozone usage	420 kg O_3 / day	440,000 kg O_3/ day
	MeOH carrier amount	13 m^3	13600 m^3
	Collected lipid	23 kg /day	24,000 kg /day
Conversion	Lipid separation tank	13 m^3	14000 m^3
	Conversion tank	13 m^3	13600 m^3
	MeOH required for reaction	21 L /day	22 m^3/day
	MeOH used for reaction	10 L /day	11 m^3/day
	H_2SO_4 used for reaction	0.4 L /day	0.4 m^3/day
	Water	650 cm^3 /day	680 m^3/day
	Biodiesel (FAME)	3300 g /day, 1 gallon /day	3,400 kg/day, 1040 gallon/day

meaningful. Therefore, it is essential that intermediate scale and pilot scale studies be conducted to determine the economic worth of the process as a commercial process.

4.9 Life cycle analysis of integrated theme process

The energy expenditures in the integrated, sequential algal harvest, rupture, and conversion are estimated. To collect by filtration after pH adjustment, the energy costs are taken from the process of pH adjustment with lime without added flocculent as shown in Table 4.8. Sand filtration after pH adjustment employed in the studied integrated process represents a great energy saving process relative to centrifugation, chamber press filtration, and pH-lime processes frequently employed for solid-liquid separation.

After algae collection by sand filtration, methanol and ozone were expended to rupture the algal biomass, followed by direct transesterification. Rupture by ozone would replace processes of drying, press, and solvent extraction. In Table 4.9, the energy expenditure for rupture was 810 kwh for an ozone dosage 50 g O_3/ kg dry algae

Table 4.8 Algae harvesting design and operational parameters to produce one functional unit (f.u.) of 10^4 MJ of algal biodiesel under economic allocation (data from Brentner et al., 2011)

Parameter	**Centrifugation**	**Chamber press filtration**	**pH-lime**	**pH-sand filtration**
Cell recovery efficiency	95%	95%	95%	95% (Sand Filtration Eff.)
Electricity use (kWh/m^3)	1	0.88	0.1	0.1
Electricity use (kWh)	2500	2200	250	250
Material use				
Polypropylene filters(kg)	-	0.15	-	-
Flocculant (kg)	-	-	750	-

pH-lime: increase of pH by lime addition
pH-sand filtration: Decrease of pH and sand filtration

Table 4.9 Lipid extraction and conversion design and operational parameters to produce one functional unit (f.u.) of 10^4 MJ of algal biodiesel under economic allocation (data from Brentner et al., 2011)

Parameter	Press + cosolvent + esterification	Ultrasonication + direct esterification	Supercritical methanol	Ozonation + direct esterification
Extraction efficiency	91%	-	-	87% (VSS Rupture Eff.)
Extraction conditions	STP	STP	-	STP (2 L/min of 1.5% O_3)
Conversion efficiency	98%	98%	98%	98%
Conversion conditions	50^{o}C	70^{o}C	250^{o}C, 8.3MPa	50^{o}C
Electricity use				
Extraction (kWh)	59	3190	-	-
Coversion (kWh)	10	-	141	10
Rupture (kWh)				810
Heat use				
Drying (MJ)	16,360	14,885	-	-
Extraction (MJ)	1000	-	-	-
Conversion (MJ)	225	400	7388	225
Reagents use				
HCl (30% vol) (kg)	1.1	403	-	1.1
H_3PO_4 (85% vol) (kg)	2.8	537	-	2.8

Press + cosolvent + esterification: Drill press to break open the plant cells followed by solvent extraction, most often with recovered and recycled hexane, followed by transesterification.
Ultrasonication + direct esterification: Direct transesterification of microalgae, with methanol added directly to dried, disrupted cells using sulfuric acid as a catalyst.
Supercritical methanol: combined lipid extraction and transesterification of oils from wet algae with a high reaction temperature (~ 250^{o}C), pumping required to supercritical pressures, and methanol recovery.
Ozonation + direct esterification: ozone rupture of the collected algae biomass in methanol and direct transesterification

(assuming 12 kwh/kg O_3, algal lipid content 20%, and 37.8 MJ/kg algal biodiesel). Ozonation and direct transesterification would replace drying and extraction in press, cosolvent, and esterification, or replace drying and extraction with ultrasonication and esterification, or replace conversion in supercritical methanol. Energy expenditures and efficiencies of various processes are shown in Table 4.8.

CHAPTER 5

CONCLUSIONS

This work demonstrated the technical feasibility of a streamlined process in obtaining algal lipid from a cultivated suspension. I conclude in the following:

5.1 Collection of agglomerated algae by sand filtration

Acidification, pH adjustment from 8.7 to 3.1, reduced repulsion among the like-charged unicellular algal cells of *Chlorococcum aquaticum*, resulting in agglomeration with particle size increase from 117.5 to 2780 nm that occurred due to neutralization of negative charges, ζ decrease from -19.2 to -5.57 mV, on the membrane surface brought by the decrease in solution pH. Sand filtration was enabled to retain the enlarged biomass. The surface potential was neutralized by lowered pH, consistent with the increased algal agglomeration brought by acidification. Sand filtration (SF) and recovery (R) efficiencies at 95 and 45%, respectively, were achieved by pretreatment in which the suspension pH was simply adjusted to 3.3.

5.2 Harvest of algal lipid and transesterification into FAME in streamlined processing

By ozonation of the algal suspension in pressure cycles, the VSS decreased by 87% with concomitant increases of VDS by 350% and sCOD by 400%. These indicated solubilization of cell materials including lipid when the membrane enclosure of algae was disrupted by ozone. Thus, processing wet algae by ozonation rupturing of the algal cells and direct transesterification of the released lipid into biodiesel compounds could replace conventional extraction and transesterification of dry algae, thus eliminating costly drying and separate steps.

While transesterification efficiencies appeared to be less overall (all solvents considered, maximum 42%) for the "wet lipid," the overall product yield (maximum 4.4%) and FAME content (maximum 51%) in the transesterified product were higher with the wet algae. Thus, the extraction and transesterification of wet algal mass without drying were viable with the hydrophilic solvent methanol.

From cells without exposure to ozone, the transesterified products were C16 to C18 containing up to 3 double bonds. When brief ozonation was applied, the transesterified products were highly saturated composed entirely of FAME compounds, albeit with a shift in abundance to smaller molecules, suggesting the occurrence of oxidation and fragmentation of the fatty acid molecule during ozonation. An optimal contact time should be determined more accurately as a long exposure was counterproductive for yield and efficiency.

5.3 Feasibility and pilot design of the streamlined process

This work demonstrated the technical feasibility of a streamlined process in obtaining algal lipid from a cultivated suspension to algal biodiesel. The new process involved acidification of the algal suspension to promote agglomeration of *Chlorococcum aquaticum*, filtration harvest of the algae, ozonation rupturing of the algal cells, and direct transesterification of the released lipid with methanol into biodiesel compounds, accomplished without the energy-intensive, separate steps of drying, grinding, and solvent extraction. Methanol addition and ozonation of the collected algae within the same filtration vessel provided a convenient method of rupturing and extraction of the algal lipid. Ozonation of the algae-loaded sand bed for 5 min was capable of both restoring the filtration velocity to its initial value (0.7 cm/min) and of removing the algal lipid from ruptured algal cells through dissolution in methanol used as an ozonation medium. Based on the finding, a streamlined system has been designed and available for further pilot testing.

In the design of an intermediate scale, three stacked sand filters require 20 m^2 of area to process 2,240 m^3 of algal suspension to be followed by ozonation, separation, and transesterification to generate 1 gallon of biodiesel per day. After testing with the intermediate scale, a pilot scale can be set up to estimate the feasibility and cost of producing 1040 gallons of biodiesel per day. The streamlined process is potentially a more efficient option of procuring lipid from cultivated algae, for which process economy is paramount when algae-derived biodiesel is contemplated as a potential source of renewable energy.

CHAPTER 6

SCIENTIFIC CONTRIBUTION AND DISSEMINATION OF RESULTS

The Phase I experiment provides an assessment of harvesting agglomerated algae by sand filtration. It is original demonstrating algae collection by sand filtration after the enabling pretreatment that neutralizes surface charge of the membrane. As a commonly practiced, inexpensive water treatment, sand filtration offers an economical, viable option for harvesting of cultivated algae.

This research also demonstrated ozonation as an effective technique to rupture algae for its lipid content, which resulted in improved purity in the produced biodiesel compounds. The results provide insights into the kinds of dissolved compounds in methanol after varied ozonation conditions and before and after transesterification.

This research proposes a viable integrated design of conventional processes with sand filtration, ozonation, and transesterification in a single vessel for greater operation efficiency. Alternatively, the released lipid in methanol can be transferred to a second vessel for further transesterification into biodiesel.

The research results contained in this dissertation are published in two papers. One entitled “A new processing scheme from algae suspension to collected lipid using sand filtration and ozonation” already appeared in Algal Research in 2013. A second

paper focuses on the effects of ozonation on products is under review and revision. It is expected to be published in 2015.

APPENDIX A

SAND FILTRATION VELOCITY UNDER DIFFERENT PH

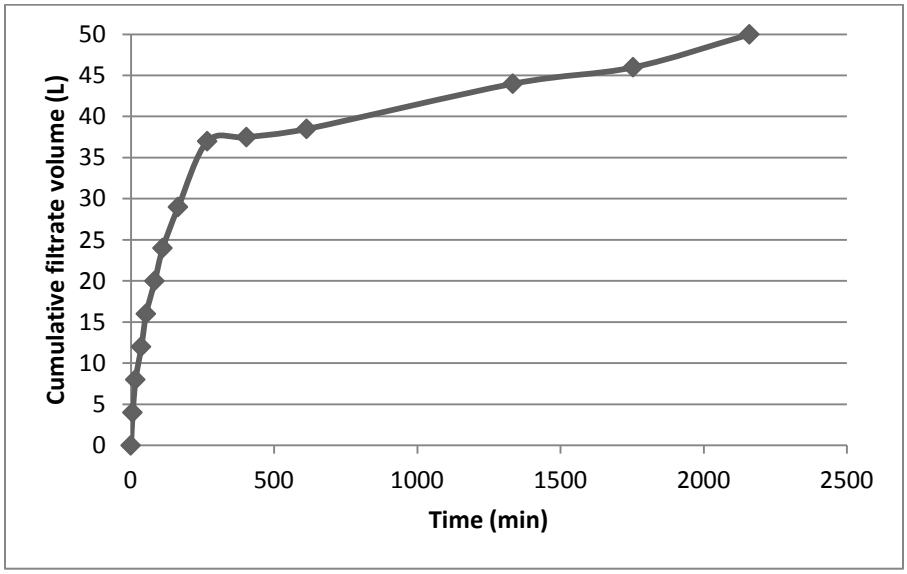


Figure A.1 Filtration velocity without pretreatment
Filtration conditions: Sand diameter, $\leqq$53 μm; depth, 21 cm; filter area, 272 cm^2; bed volume, 5712 cm^3; initial filtration velocity, 24.5 L/m^2-min; final filtration velocity, 0.36 L/m^2-min;over decreasing hydraulic head from 12 cm above bed.
Influent concentration to sand bed (mg/L) = 304, 284, 838, 344 for TSS, VSS, TS, and VTS, respectively
Sample volume: 50 L (# of bed volume= 8.75)

Without pH adjustment, the filtration velocity decreased abruptly after 35 L, suggesting that algal biomass can be trapped in the sand column and that the column must be regenerated before filtration collection can continue.

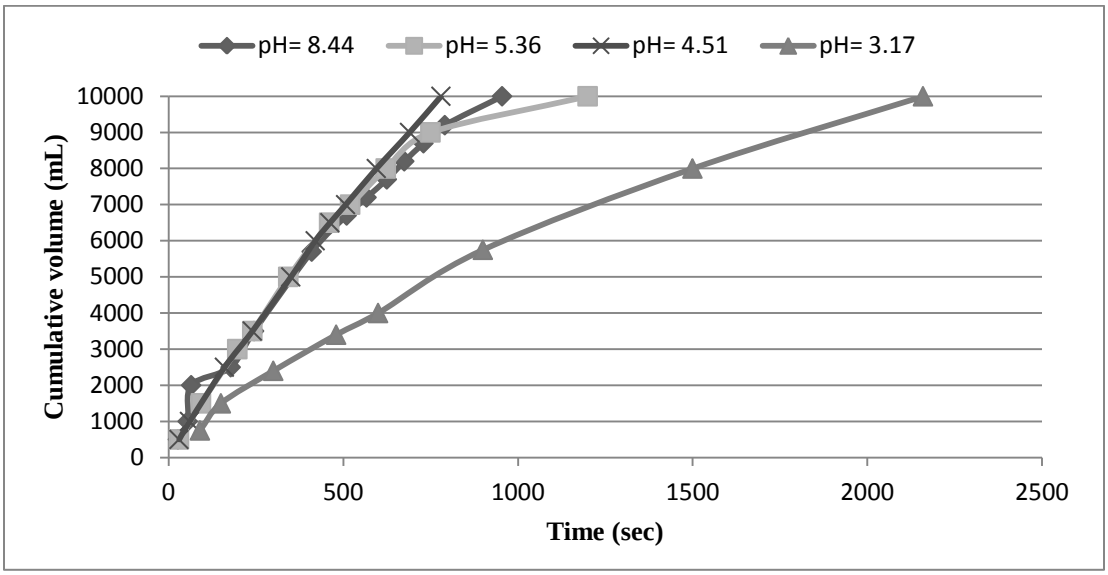


Figure A.2 Filtration velocity under different pH

Filtration conditions: Sand diameter, $\leqq$53 μm; depth, 21 cm; filter area, 272 cm^2; bed volume, 5712 cm^3; filtration velocity, 10.2-28.2 L/m^2-min; over decreasing hydraulic head from 12 cm above bed.
Original algae concentration (mg/L) = 145-220, and 126-190 for TSS, and VSS, respectively
Sample volume: 10 L (# of bed volume= 1.75)

From Figure A.1, the algae would be entrapped in the sand column under extended operation. During operation, a part of the algae biomass still appeared in the effluent, especially those of small particles. After extended operation, the entrapped algal biomass would form a filter cake on the top of the sand column which would help to trap all algal biomass. But the sand filter was near inoperable at the stage of cake formation. So pH adjustment was introduced to promote growth of algal aggregates. In Figure A.2, the filtration velocity steadily decreased when the pH was adjusted to 3.2. At pH 3.2, sand filtration proceeded effectively in algae collection.

APPENDIX B

CHARACTERISTIC OF OZONATED ALGAE SAMPLE

Table B.1 Changes of algae samples subjected to varied O_3 contact times

Sample	Untreated	2.5 min	3 min	3.5 min	4 min
TSS (mg/L)	183	115	104	106	144
VSS (mg/L)	175	108	94	102	118
TS (mg/L)	792	722	704	720	722
VTS (mg/L)	348	278	274	244	260
Total soluble solid (mg/L)**	609	607	600	614	578
Volatile soluble solid (mg/L)***	173	170	180	142	142
COD (mg/L)	385	282	276	303	262
SCOD (mg/L)	51	81	76	83	79

Ozonation conditions: 1.5% O_3 at 2 L/min by a diffuser into 1 L of algae sample with stirring. Dosages for 2.5, 3, 3.5, and 4 minutes are 815.3, 978.3, 1141.4, and 1304.4 mg O_3/g TSS algae, respectively.

Changes of water characteristics showed optimal ozonation time at 3 min, when TSS and VSS decreased from 183 mg/L and 175 mg/L to 104 mg/L and 94 mg/L, respectively, while VDS decreased from 173 mg/L to 180 mg/L.

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