International Journal of Engineering and Technical Research (IJETR)
ISSN: 2321-0869, Volume-2, Issue-11, November 2014

In-situ Transesterification of *Chlorella vulgaris* towards Bio-Jet Fuel Production

Marian Elmoraghy, Ihab H. Farag

***Abstract*— The increase in petroleum-based aviation fuel consumption, the decrease in petroleum resources, the fluctuation of the crude oil price, the increase in greenhouse gas emission and the need for energy security are motivating the development of Bio-jet fuel, an alternate jet fuel. Bio-jet fuel has been produced by blending petro-based jet fuel with *Chlorella vulgaris* biodiesel (Fatty Acid Methyl Ester, or simply FAME). Microalgae lipids extraction and transesterification to biodiesel are energy and time-consuming (roughly 5 hours in the lab). This study investigated the integrated one-step extraction/ transesterification of freeze dried *Chlorella vulgaris* to produce biodiesel for blending with jet fuel. The one step or "in-situ" transesterification reaction was run in methanol assisted by ultrasonication, and was completed in 30 minutes. The FAMEs produced were identified using a gas chromatograph. Yields up to 56.82 mg FAME/g dry algae were obtained. Predicted physical properties of in-situ FAME satisfied European and American standards confirming its quality.**

***Index Terms*— *Chlorella vulgaris*, microalgae, biodiesel, in-situ, Oil Extraction, Ultrasound, Fatty Acid Profiles.**

I. INTRODUCTION

A. Airline Industry Challenges.

The airline industry enjoys a yearly passenger ridership of over 2.2 billion and delivers over 30% of all international material. Estimates are that over 32 million jobs are directly related to the US$3.5 billion airline industry. However, the industry is confronted with environmental and financial challenges. These include increasing petroleum-based jet fuel price (which tripled in the last 7 years), dependency on imported petroleum oil and deteriorating climate due to global anthropogenic greenhouse gas (GHG) emission. The industry currently generates about 2% of the GHG. This is estimated to reach about 3% by 2050. It is highly desirable to reach a carbon-free airline industry.

B. Bio-Jet Fuel.

The airline Renewable aviation fuel, also known as bio-jet fuel is the most desired alternative to replacing carbon-intensive petro-based jet fuel. Bio-jet fuel is a drop-in alternative fuel; less dependent on and greener than petroleum-based jet fuel; has low volume per unit energy (i.e., has a low gallon per Btu); and could reduce flight-related GHG emissions by over 60% compared to petroleum-based jet fuel. The renewable aviation fuel can be produced domestically from local resources, thus, it provides the airline industry a secure supply of liquid fuel. In addition, bio-jet fuel is produced from sustainable biomass like microalgae.

Algae are aquatic organisms that contain green pigments (chlorophyll) in the cells. The chlorophyll uses photonic energy (light), carbon dioxide (CO2) and water to synthesize a number of chemicals, e.g., lipids. They do not compete for arable land and can be produced year round. Microalgae are small microscopic aquatic photosynthetic plants (around micrometers). They are single celled that grow quickly in water suspension. They use light energy to obtain their inorganic compound's nutritional needs. Some microalgae contain large amounts of lipids within their cells. The ideal natural oil feedstock for biodiesel and bio-jet production are of triacylglycerides (TAGs), [1], [2], [3]. Figure 1, shows the structure of a TAG.

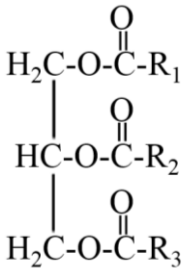

Fig. 1 Structure of triacylglyceride (TAG). R_1-COOH, R_2-COOH and R_3-COOH in the TAGs are saturated and unsaturated fatty acids. These could be short or long chains hydrocarbons. Shorter chain length fatty acids (16-21 carbon atoms) are ideal for making biodiesel

The lipids (TAGs) content of microalgae is usually between 10-50 g per 100 g dry algae. Table 1 gives a list of some saturated (S) and unsaturated (U) FAs found in algal cells. The labels S and U are listed in the first column. The second column contains the name of each fatty acid (FA) is followed by the total number of carbon, and total number of double bonds; for instance, (16:1) indicates FA (palmitoleic or sapienic) of 16 carbons with one double bond. The chemical abstract service registry (CAS) number is listed in the third column. The formula is given in column 4. Algae cells of each strain do not contain every single fatty acid displayed in Table I. The lipids FAs should contain 16 – 21 carbons for biodiesel production. The resulting biodiesel will have flash point range of 120°C to 150°C. This satisfies the European standards of biodiesel flash point greater than 101°C (the flash point range of petroleum diesel is 60°C to 90°C). The flash point is the temperature at which a vapor can be ignited in air. Hence, biodiesel has lower fire risk than petroleum diesel.

Manuscript received October 28, 2014.
Marian Elmoraghy, Chemical Engineering Department, University of New Hampshire, Durham, New Hampshire, USA,
Ihab H. Farag, Chemical Engineering Department, University of New Hampshire, Durham, New Hampshire, USA, +1-603-767-4442.

TABLE I
FATTY ACIDS (FAS) FOUND IN ALGAE CELLS. [4], [5], [6]

	FA Name/ Abbreviation	CAS No.	Formula
S	Palmitic (16:0)	57-10-3	CH_3-$(CH_2)_{14}$-COOH
U	Palmitoleic (16:1)	373-49-9	$CH_3(CH_2)_5$**CH=CH**$(CH_2)_7$COOH (= bond at C7)
U	Sapienic (16:1)	17004-51-2	$CH_3(CH_2)_8$**CH=CH**$(CH_2)_4$COOH (= bond at C10)
U	Hexadecadienoic (16:2)	25377-52-0	$CH_3(CH_2)_{10}$**CH=CHCH=CH**COOH
U	Hexadecatrienoic (16:3)	25377-56-4	$CH_3(CH_2)_4$**CH=CH**CH2**CH=CH**CH2**CH=CH-**$(CH_2)_2$COOH
S	Stearic (18:0)	57-11-4	CH_3-$(CH_2)_{16}$-COOH
U	Oleic (18:1)	112-80-1	$CH_3(CH_2)_7$**CH=CH**$(CH_2)_7$COOH
U	Linoleic (18:2)	60-33-3	$CH_3(CH_2)_4$**CH=CH**CH_2**CH=CH**$(CH_2)_7$COOH
U	α-Linolenic (18:3)	463-40-1	CH_3CH_2**CH=CH**CH_2**CH=CH** CH_2**CH=CH**$(CH_2)_7$-COOH
U	Octadecatetraenoic (18:4)	25448-06-0	CH_3CH_2**CH=CH**CH_2**CH=CH** CH_2**CH=CH**CH_2**CH=CH**$(CH_2)_4$-COOH
S	Arachidic (20:0)	506-30-9	CH_3-$(CH_2)_{18}$-COOH
U	Arachidonic (20:4)	506-32-1	$CH_3(CH_2)_4$**CH=CH**CH_2**CH=CH**CH_2**CH=CH**CH2-**CH=CH-**$(CH_2)_3$COOH
S	Behenic (22:0)	112-85-6	CH_3-$(CH_2)_{20}$-COOH
U	Eicosapentaenoic (20:5)	10417-94-4	CH_3CH_2**CH=CH**CH_2**CH=CH** CH_2**CH=CH**CH2-**CH=CH**CH_2**CH=CH(**CH_2 $)_3$COOH

Table II lists the FAs distribution in vegetable oils used in the production of biodiesel. All FAs contain saturated fatty acids (C16:0 and C18:0) and unsaturated fatty acids (C18:1, C18:2 and C18:3). The ratio of [total average unsaturated fatty acids (C16:1 + C18:1 + C18:2 + C18:3)/total average saturated fatty acids (C16:0 + C18:0)] is abbreviated as "Ratio U:S FA" and included in the last column of Table II.

TABLE II

FA Distribution in Vegetable Oils Used to Produce Biodiesel

Oil	Density kg/m^3	Viscosity mm^2/s	Flash Point, °C	C16:0 (Avg) %	C18:0 (Avg) %	C18:1 (Avg) %	C18:2 (Avg) %	C18:3 (Avg) %	Ratio U:S FA
Soybean	914	32.6	254	6-10 (8)	2-5 (3.5)	20-30 (25)	50-60 (55)	5-11 (8)	88/11.5= 7.65
Corn	922	65	254	8-12 (10)	2-5 (3.5)	19-49 (34)	34-62 (46)	Trace	80/13.5= 5.93
Hi Oleic Rapeseed	911	37	246	4.3	1.3	59.9	21.1	13.2	94.2/5.6 =16.8

The length of the hydrocarbon chain and the degree of unsaturation influence the heating value, viscosity, cloud point, lubricity and pour point of the biodiesel product. [7], [8]. Increasing saturated FAs (or decreasing unsaturated FAs) decreases NOx emissions, improves oxidative stability and long term storage and reduces deposition, but it increases melting point and viscosity and reduces lubricity. The oil viscosity is a measure of the resistance to flow and the thickness of the oil layer on a metal part. The lubricity refers to the actual slipperiness of the lubricant. Some *Chlorella* strains contain only C16s and C18s [4], making it suitable for biodiesel production. This work focused on the use of *Chlorella vulgaris* to produce biodiesel which is then blended with jet fuel to produce bio-jet fuel.

C. Bio-Jet Microalgae to Bio-Jet Fuel Steps.

It is important to understand the steps to produce bio-jet fuel from microalgae to run an efficient process. The approach taken in this research is to first produce biodiesel which is then blended with jet fuel to produce bio-jet fuel. Considerable research has been done to study the production of biofuels [9], [10], and specifically biodiesel from vegetable oils [5], [11], [12], [13] , and the characterization of the biodiesel properties [11], 12], [14]. Further research focused on the use of algae to produce the oil needed to obtain biodiesel [6], [15], [16]. Algae triggering was studied to improve the microalgae oil yield [17], [18]. Several investigators looked at the use of municipal wastewater for algae growth [19], [20] , [21], [23], [24], the offshore growth of algae [25], the use of natural sunlight and carbon dioxide [12], [22], marine algae [4], and the kinetics of the process [8], [26] to improve the economics. Figure 2 summarizes the steps to produce microalgae biodiesel and bio-jet fuel from microalgae [22], [23], [24]. The steps required to obtain microalgae oil include microalgae cultivation in water and nutrients in the presence of photonic (light) energy, harvesting, dewatering, freeze-drying to produce dry algae, and oil (lipids, TAGs) extraction. The extracted oil is transesterified with methanol and a catalyst to liquid biodiesel (FAME) fuel. When algae are cultivated indoors, fluorescent light and fresh water are often used. After harvesting and drying, the oil is extracted with n-hexane, then hexane is evaporated and condensed for reuse. The recovered oil becomes the feedstock to produce biodiesel which is then blended with petroleum jet fuel to produce bio-jet fuel.

Fig. 2 Steps in the manufacture of Bio-Jet fuel from Microalgae. The transesterification reaction is shown under the transesterification step.

D. In-Situ Transesterification.

Microalgae biodiesel production includes two steps; lipid extraction and the transesterification of the extracted lipid to biodiesel. Solvent extraction of lipids from microalgae for biofuels production is time-consuming and requires additional energy to evaporate the chemical solvent. In-situ (one-step process, or direct transesterification) is a single-step integrated process and is one way around these problems. In-situ refers to the direct transesterification of the lipids [6], [15], [22], [27], omitting the need for an initial lipid extraction. Oil-bearing dried *Chlorella vulgaris* microalgae

are sonicated to crack the algae outer shell. Then the microalgae are reacted directly with alcohol and catalyst, thereby eliminating the need for pre-extracted oil, and its associated capital and intensive running costs. Advantages of the in-situ process include: one step for integrated lipid extraction and transesterification of lipids to biodiesel; No need for hazardous chemical extraction solvents, like hexane, reduced processing time; production and recovery of FAME can be done within 90 minutes [28], [29]. Among the disadvantages, the sonication time has significant effect on the FAME content as it affects the extraction and transesterification. The sonication increases the temperature and hence, improves the methanol extraction of microalgae oil. Longer sonication times, i.e., above 10 minutes are inefficient as they may result in overheating of the reaction mixture and more losses of the methanol and the biodiesel [15], [22].

E. Goal.

There are considerable investigations on reducing the production time and cost of microalgae biodiesel, the blending of biodiesel and petroleum-based jet fuel to produce bio-jet fuel. Clearly improvements in the bio-jet production process that would decrease production time and the fresh water used, lower the energy use and make the process greener are highly desirable. For bio-jet production, additional requirements are to obtain a liquid fuel with desirable characteristics, e.g., freezing point, volume per unit energy, heat of combustion and viscosity. The goal of this project is to develop an economical process for sustainable microalgae bio-jet fuel production. The economics of bio-jet fuel and algae oil production can be improved using the in-situ process and possibly using municipal waste water and efficient light source for algae growth.

II. Materials and Methods

The steps taken to accomplish the above goals are to: investigate *Chlorella vulgaris* algae growth and harvesting; extract algae lipid using solvent; produce biodiesel by conventional/two-step and by the in-situ/one-step processes; use gas chromatography to determine the Fatty acids composition of the biodiesel produced from *Chlorella vulgaris*, determine the effect of the growth medium water source (fresh vs municipal waste water), and of the light energy source (fluorescent v.s. light emitting diodes, or LEDs) on the biodiesel yield and use the biodiesel fatty acids composition to predict its physical properties.

A. Algae Harvesting

The algae nutrient solution was prepared by dissolving the nutrient chemicals in fresh water or municipal waste water. The municipal waste water used was collected from a local waste water treatment plant after ultra-violet (UV) treatment. This insured that absence of pathogens and the safety of the lab staff. Typical properties of the waste water used are pH=6.83, nitrate nitrogen (NO_3) = 7.5 mg/l, ammonia nitrogen (NH_3) = 6 mg/l, total nitrogen (TN) =14 mg/l, total phosphate (TP) =1.3 mg/l, biological oxygen demand (BOD) =10 mg/l and total suspended solids (TSS) =7 mg/l. The nutrient solution used provided 72 mg N/l and 12.4 mg P/l and about 0.041% of the C in the air. Hence, the variation of waste water content on algae growth was negligible.

The algae were grown at room temperature in a 2 liter batch photobioreactor (PBR). Algae aliquot and nutrient solution were placed in the indoor PBR and exposed to the light source (Fluorescent light, red LEDs and red-blue LEDs). Ambient room-temperature air was continuously bubbled in the PBR at a rate of 2 Liters/min to agitate and provide CO_2 to the algae solution during the growth period. Algae were harvested, dewatered and freeze-dried to produce the dry algae. Figure 3 shows the experimental setup.

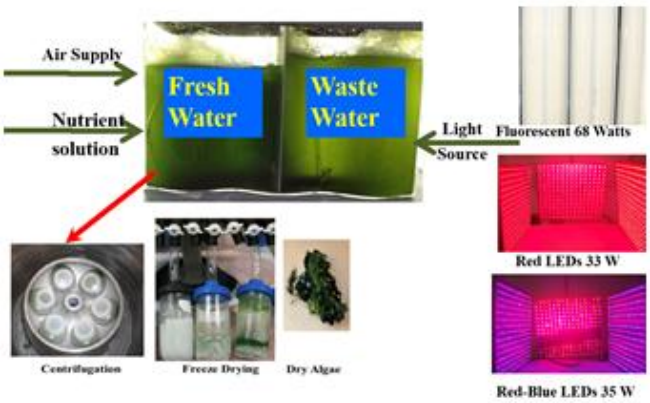

Fig. 3 Experimental Setup. Algae was grown in two-liter photobioreactors. The photobioreactor vessel was a four liter clear plastic tank, divided into two separate 2 L PBRs and operated at the same time. Three different light sources were tested, fluorescent, red LEDs and red-blue LEDs. Microalgae were harvested in fresh and municipal waste water. Once cultivated, the algae were centrifuge dewatered and freeze dried to produce dry algae.

B. Solvent Lipid Extraction

The next step is to extract the microalgae oil/lipids from the dry algae using hexane solvent. Figure 4 shows that the algae hexane mixture is filtered to separate the left-over algae. Next, hexane is evaporated and the dry microalgae oil is collected for transesterification to biodiesel, which is blended with jet fuel to produce bio-jet fuel.

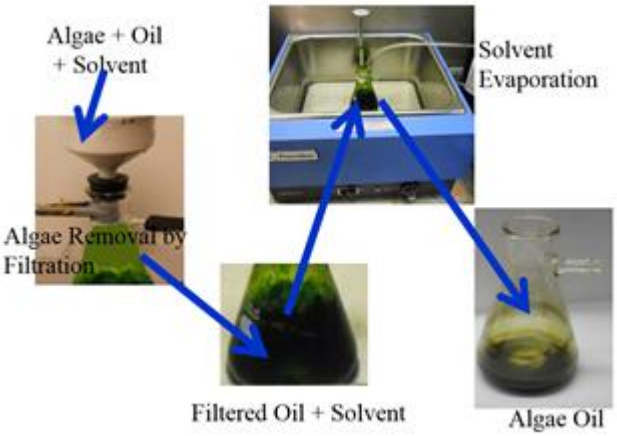

Fig. 4 Algae removal and recovery of extracted algae oil from the solvent.

C. Conventional (Two-Step) Transesterification

The conventional transesterification process to produce biodiesel consists of two steps; the first is to extract the lipids from algae cells and the second is to transesterify the extracted lipids into biodiesel. The extracted lipid is mixed with a 0.1 N KOH solution in methanol and heated at 50°C for

30 minutes. The excess methanol was then evaporated. The remaining transesterified oil was dehumidified using anhydrous sodium sulfate. Then, Chloroform was added to dissolve the produced FAMEs. This final product was transferred to a glass vial, ready for the gas chromatograph (GC) analysis to determine the FAME yield and distribution.

D. In-Situ (One-Step) Process

While the two-step process is used industrially, it is desirable to find a less time-consuming process. Moreover, the n-hexane solvent used for lipid extraction is expensive and hazardous. Industrially, the hexane is recovered and reused, but it would be desirable to eliminate its use. The one-step process (in-situ transesterification) does not use any hazardous or expensive extraction solvent and takes only 30 minutes in the lab. Figure 5 shows a schematic of the two-step versus the one-step processes.

The starting material was dry algae. A W375 ultrasonicator was used to break the algae cell membrane and release algae oil. The transesterification reaction took place at the same time.

Pulverized algae were mixed with 0.1 KOH in methanol and the mixture was sonicated at a power density of 9.4 KW/L. The effect of the sonication and reaction duration (time) on the FAME yield was studied. After the reaction is completed, the remaining algae particles were removed by filtration. Excess methanol was evaporated from the filtered biodiesel fuel using a 40°C water bath and air. The biodiesel fuel was mixed with chloroform to get it ready for the GC analysis.

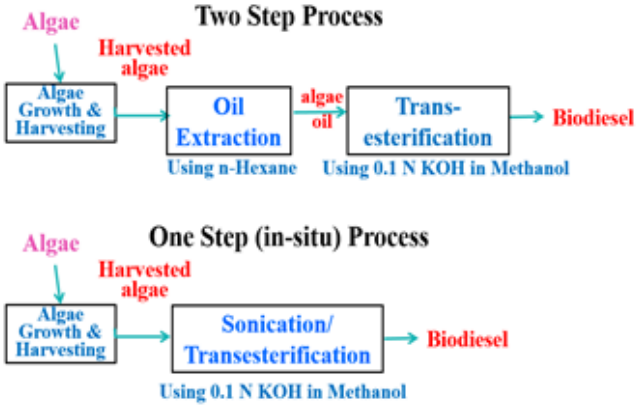

Fig. 5 Biodiesel production; the one-step versus the two-step processes.

E. Gas Chromatograph (GC) Analysis

A Hewlett Packard HP 5890 Series II Gas chromatograph (GC) was used to identify the biodiesel FAMEs. The GC consisted of (1) an oven, which contained an RTX fused silica fast column 30 m long, 5.0 µm film thickness and 0.32 mm inner diameter, (2) a cool-on column injection port used to inject the FAME sample, (3) a flame ionization detector (FID) supplied with hydrogen gas to ignite the flame. The GC oven temperature started at 240 °C and was kept for 2 minutes, then ramped from 240 °C to 275°C at 15°C /min. The 275°C final temperature was held for 10 minutes. The injection temperature was 275°C and the detection temperature was 280°C. Helium was used as the carrier gas, with a flow rate of 1.34 mL/min or about 24.5 cm/s at 50°C. A 3µL sample of the FAME in chloroform solution was injected into the cool-on column using a 5 µL Hamilton syringe. The GC FID detector generated a voltage signal as it detected each FAME component. The total GC run period was 25 minutes. The GC was connected to a Hewlett Packard (HP) 3396 series II integrator to process the GC FID detector analog signal and plot it into a visual printout (chromatogram). It also determined the integrated area under each peak and compared it to a reference/standard peak. The integrator was interfaced with a PC computer running the HP PEAK96 software. It was used to transfer the data files from the HP 3396 series II integrator to the PC, integrate the peak areas and save the data as an Excel file for data analysis, as shown in Figure 6.

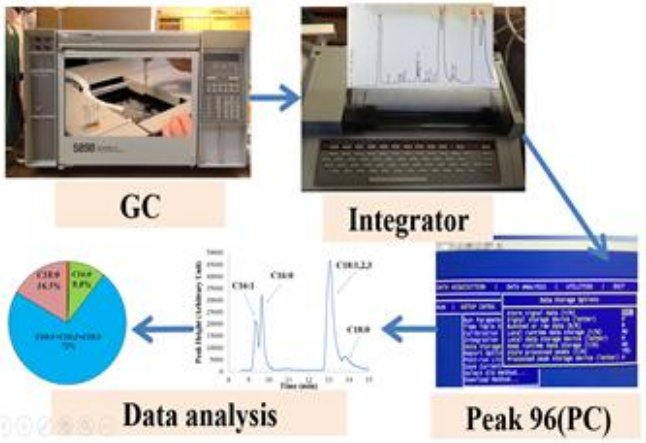

Fig. 6 Fatty Acids analysis using the HP Gas Chromatograph (GC) 5890 Series II. The sample is injected into the GC and the run is started. The HP 3396 integrator collects the GC data, plots the chromatograms and calculates the areas under each peak. Peak 96 collects the data from the integrator and saves as files on the PC. The files are analyzed in excel to draw the chromatograms and calculate the FAME yield and the percent of each fatty acid (FA). These FA percentages are plotted as a pie chart.

Biodiesel FAMEs were identified and quantified by the following steps. Analytical reference standards C16:0, C16:1, C18:0, C18:1, C18:2 and C18:3 were prepared to known concentrations. Each standard with its known concentration was injected in the GC individually to determine its retention time and the integrated area of the identified peak. Two different cocktails of FAME standards were prepared. Each cocktail contained a mixture of the above reference standards. Each cocktail was injected in the GC to identify FAME peaks and confirm retention time. The area under each peak is proportional to the corresponding concentration of the same standard. This procedure was used to calculate the concentrations of C16:0, C16:1 and C18:0 in the biodiesel sample. The three unsaturated C18:1, C18:2 and C18:3 had overlapped peaks. The peaks of a biodiesel sample could be identified by comparing their retention time to the retention time of the standards when injected individually. Any peak that has a retention time different from the known standards was considered an unknown peak. The total FAME yield per gram of dry algae was calculated by multiplying (the sum of the resulted concentration of C16:0, C16:1, C18:0, C18:1, C18:2 and C18:3, gm/ml), times (the total volume of biodiesel and chloroform, ml). One gram of dry algae was used for each in-situ or conventional transesterification. The results were presented as pie charts of the fatty acid distribution.

F. Biodiesel Physical Properties Estimation

The chemical structure of petroleum diesel fuel is a saturated non-branched hydrocarbon molecule with carbon in the range of C12:0 to C18:0. Biodiesel FAMEs tend to exhibit

a greater degree of unsaturation compared to petroleum diesel fuel. It is important to know thermo-physical properties like density and kinematic viscosity. For combustion purposes, it is important to know properties like heat of combustion and the Cetane number. One of the most important properties is density (or specific gravity). When blending biodiesel with jet fuel to produce bio-jet fuel it is important to realize that biodiesel is denser than diesel. There is often an upper limit of the density of the jet fuel. Knowledge of the biodiesel density is crucial to making sure the blend does not exceed the specifications of jet fuel. The Cetane number is an important biodiesel property, but its measurement is not a simple process and is time-consuming. Accurate knowledge of biodiesel FAMEs composition would permit the use of estimation methods available in the Literature. Four empirical correlations were provided to calculate the Cetane number, kinematic viscosity, density and higher heating value of the FAMEs [30]. The correlations related the properties of the FAMEs to their molecular weight and the degree of unsaturation. The Cetane number Φ_i of the i_{th} FAME is obtained from Equation 1.

$$\Phi_i = - 7.8 + 0.302\ M_i - 20\ N \qquad (1)$$

Where M_i is the molecular weight of the i_{th} FAME and N is the degree of saturation, i.e., the number of double bonds (DB) in a given FAME. The natural log of the kinematic viscosity ν_i (at 40°C of the i_{th} FAME in mm^2/s) as a function of M_i and N is expressed in Equation 2.

$$\ln(\nu_i) = -12.503 + 2.496 \ln(M_i) - 0.178\ N \qquad (2)$$

The density ρ_i at 20 °C of the i_{th} FAME in g/ml is expressed in Equation 3.

$$\rho_i = 0.8463 + 4.9/M_i + 0.0118\ N \qquad (3)$$

The higher heating value of a FAME, δ_i in KJ/g can be calculated from Equation 4.

$$\delta_i = 46.19 - 1794/M_i - 0.21\ N \qquad (4)$$

The physical properties of biodiesel can be estimated from the individual physical properties of its FAMEs using appropriate mixing rules [30], i.e.,

$$f_b = \Sigma_i\ z_i\ f_i \qquad (5)$$

Where f is a function that represents any physical property (the subscript b and i refer to the biodiesel and the pure i_{th} FAME, respectively), z_i is the mass fraction of the i_{th} FAME.

III. Results and Discussion

A. Conventional Transesterification FAME Yield

Dry *Chlorella vulgaris* algae from the same batch were used to compare the FAME production of the two-step and the one-step process. Figure 7 shows the *Chlorella vulgaris* biodiesel FAME composition produced in the present work by the traditional two-step process. The major FAMEs produced are C18:1,2,3 (42.7%). The total unsaturated FAMEs (C16:1 + C18:1,2,3) is 55.2% and the total saturated FAMEs (C16:0 +C18:0) is 32.6%. The ratio of [total average unsaturated FAs (C16:1 + C18:1 + C18:2 + C18:3)/total average saturated FAs (C16:0 + C18:0)] is 1.69, which is lower than the vegetable oils of Table 2.

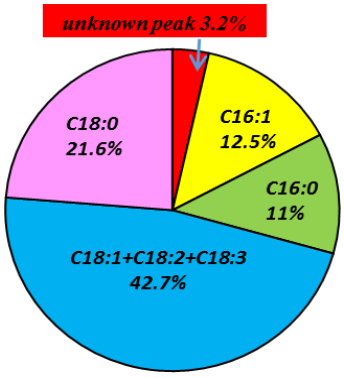

Fig. 7 Composition of Chlorella vulgaris biodiesel FAMEs produced in the two-step process. The unknown peaks are about 3.2%.

B. Optimum In-situ Time for FAME Yield

The In-situ process produces biodiesel through combined algae oil extraction and transesterification. The process sonicated a mixture of algae and 0.1 M KOH in methanol. The effect of the (sonication and reaction) time on the biodiesel FAME production was studied in order to determine the optimum time as shown in Figure 8.

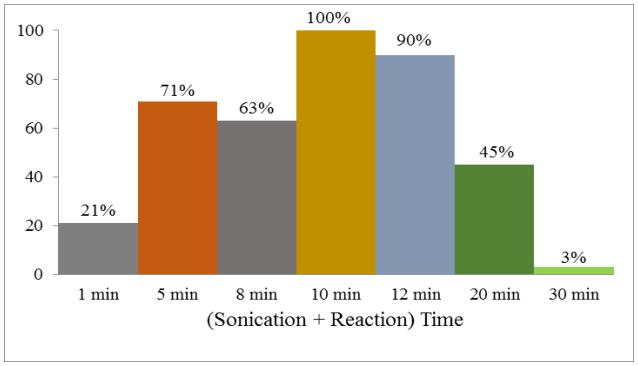

Fig. 8 Relative Chlorella vulgaris in-situ total FAMEs concentration compared to the optimum (Sonication + Reaction) time. Runs conditions: *Chlorella vulgaris*, fresh water medium and using fluorescent light.

The total FAME concentration increases with time until the optimum sonication and reaction time, 10 minutes, is achieved at which maximum total FAME concentration was obtained. When the time increased more than 10 minutes, the total FAME concentration decreased. The reason is that the sonicator releases so much heat which might destruct the FAMEs (C16 – C20) to smaller molecules (C8-C14), which are not detected by the GC.

C. In-Situ Chlorella vulgaris biodiesel FAME Yield

3 µL of *Chlorella vulgaris* biodiesel produced in the one step process was injected in the GC and the data was analyzed. The FAMEs composition of the *Chlorella vulgaris* biodiesel produced in the one-step was obtained. The total FAMEs are; unsaturated 68.3% and saturated 29.3%, with U:S of 2.33. The results are compared to the two-step process in Figure 9.

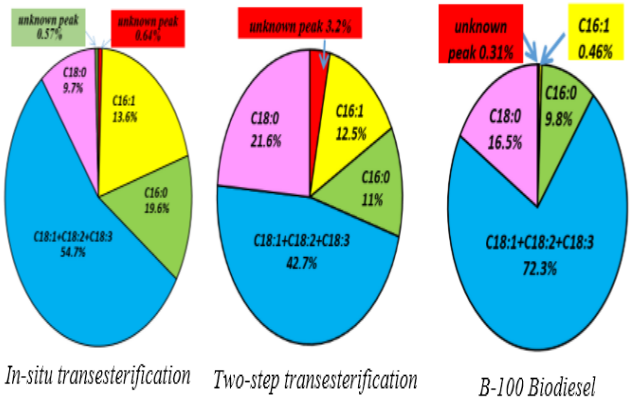

Fig. 9 Composition of *Chlorella vulgaris* biodiesel and B-100 biodiesel FAMEs comparison. Left side, FAMEs produced in the one-step process, with unknown peaks of about 1.2%. Middle chart, FAMEs produced in the two-step process, with unknown peaks of about 3.2%. Right Side, B-100 biodiesel FAMEs, with unknown peaks of about 0.31%.

Figure 10 shows that Chlorella vulgaris biodiesel FAME composition produced in the one-step process is comparable to that in the two-step process. The U:S FA is 2.37 for the in-situ biodiesel versus 1 for the two-step process. The absence of hexane solvent might have caused the FA content change.

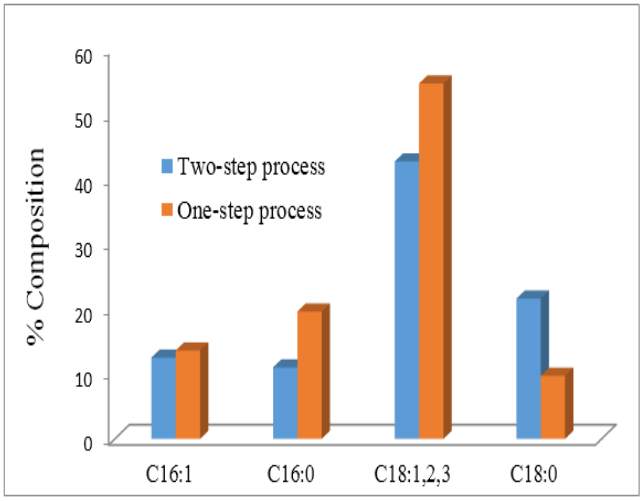

Fig. 10 Comparison of Chlorella vulgaris FAMEs compositions produced by the traditional two-step and the one-step processes.

The effect of the oil feedstock source on the FA composition of the biodiesel product was studied as this affects the properties of the biodiesel. Pure biodiesel (B100) from waste vegetable oil, produced by White Mountain Biodiesel, LLC, was analyzed using the same HP 5890 Series II gas chromatograph. 1 ml of the B100 was diluted with chloroform at a ratio of 1:100 by volume. 3 µL of the diluted B100 was injected in the GC. Total FAME concentration was 138.06 mg FAME/ml of B100. Figure 9 compares the FAMEs composition of B100 biodiesel to the in-situ and two-step *Chlorella vulgaris* biodiesels. The waste oil biodiesel tends to have a higher content of C18 and lower C16 than the *vulgaris* biodiesel fuel.

D. Effect of Water and Light Sources on In-situ FAME Yields

Figure 11 shows the one-step average *Chlorella vulgaris* total biodiesel FAME yield. The total FAME yield of fresh water and Red-Blue LEDs (18.13 mg FAME/g dry algae) is 33% of that of fresh water, Fluorescent (55.52 mg/g of dry algae) for *Chlorella vulgaris*, which indicates that Red-Blue LEDs is comparable to Fluorescent in large scale PBRs since the light intensity of the Red-Blue LEDs jacket is one fourth of the Fluorescent light intensity. However, waste water with the low light intensity of the Red-Blue LEDs for *Chlorella vulgaris* produce low FAME yield (0.59 mg FAME/g of dry algae).

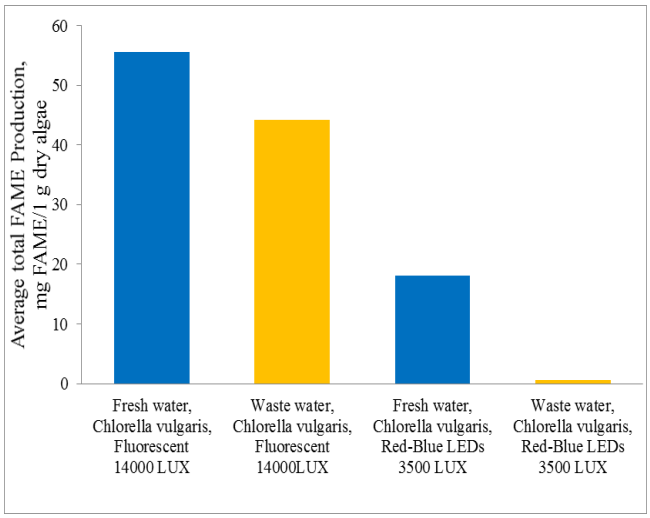

Fig. 11 Effect of light and medium sources on algae total FAME yield.

E. Biodiesel Physical Properties Estimation

Equations 1 to 4 were used to predict the properties of each pure FAME. The results are listed in Table III. Comparing the kinematic viscosities of (C16:0 and C16:1) and of (C18:0, C18:1, C18:2 and C18:3) confirm earlier statements that the increase in the saturated FAMEs causes the viscosity to increase.

TABLE III
PREDICTED PHYSICAL PROPERTIES OF PURE INDIVIDUAL BIODIESEL FAMES

FAME	Mol. weight (M_i)	N	20 °C ρ_i g/ml	ν_i 40 °C mm^2/s	Cetane no. Φ_i	δ_i KJ/g
C16:1	268.43	1	0.8764	3.589	53.267	39.297
C16:0	270.45	0	0.8644	4.366	73.876	39.557
C18:1	296.49	1	0.8746	4.599	61.739	39.929
C18:2	294.47	2	0.8865	3.785	41.131	39.678
C18:3	292.46	3	0.8985	3.114	20.522	39.426
C18:0	298.50	0	0.8627	5.590	82.348	40.180

Equation 5 was used to predict the physical properties of the produced biodiesel. The results of four different batches are shown in Table IV, together with the ASTM 6752 and EN14214 biodiesel standards.

TABLE IV
PREDICTED PHYSICAL PROPERTIES OF ALGAE BIODIESEL PRODUCED IN THE ONE-STEP PROCESS AND THE BIODIESEL STANDARDS (ASTM AND EN14214)

Batch	ρ_b g/ml 20 °C	M_b g/gmol	ν_b mm^2/s 40°C	Φ_b Cetane no.	δ_b KJ/g
1A1	0.878	290.89	4.158	54.202	39.7
1B1	0.877	290.73	4.261	57.083	39.8
1C3	0.876	291.68	4.323	58.038	39.8
1D3	0.879	291.44	4.122	52.841	39.7
Biodiesel Standards					
ASTM 6752	0.860 to 0.900		1.9 to 6.0	≥ 47	38 to 45
EN 14214			3.5 to 5.0	≥ 51	≥ 35

The predicted physical properties, i.e., density, kinematic viscosity, Cetane number and heat of combustion, of the produced biodiesel satisfy EN14214. This is the European Standard that describes the requirements and test methods for biodiesel fuels. EN 14214 standards are either the same or tighter than the ASTM 6752. This is the US Standard that provides standards and specifications for biodiesels fuels. Therefore, a biodiesel sample that satisfies the density, viscosity, kinematic viscosity, Cetane number and higher heating values of the European Standards EN 14214 will also satisfy ASTM 6752.

IV. CONCLUSIONS

The in-situ biodiesel showed higher FAME yield (55.52 g FAME/one g dry *Chlorella vulgaris* algae) than two-step process (4.03 mg FAME /g dry *Chlorella vulgaris* algae). In addition, the in-situ process takes less time in the lab and does not use hazardous and expensive n-hexane solvent. It has also been shown that the municipal waste water is a promising medium for algae growth thus, conserving fresh water. Waste water medium, with nutrients produced 80% of the total FAME of fresh water with nutrients for *Chlorella vulgaris*. Red-blue LEDs are promising low-energy replacements to Fluorescent lights in large scale PBRs. The predicted product biodiesel properties of density (0.8788 g/mL) and heat of combustion (39.81 KJ/g) were comparable to the present work's measured density (0.853 g/mL) and heat of combustion (40.82 KJ/g). The predicted density, kinematic viscosity, Cetane number and higher heating value satisfied the European and US standards.

ACKNOWLEDGMENT

The authors thank Dr. Leland Jahnke for assistance with microalgae preparation and growth and for stimulating discussion and Dr. Nancy Whitehouse, UNH Dairy Research Center, for help with centrifugation and freeze drying.

REFERENCES

[1] Y. Chisti, "Biodiesel from microalgae," *Biotechnology Advances*, **2007**, 25, 3, 294-306.
[2] Y. Chisti, "Biodiesel from microalgae beat bioethanol", *Trends in Biotechnology* **2008**, 3, 126-131.
[3] O, Danielo, "An algae-based fuel." *Biofutur*, **2005** (255).
[4] M. Matsumoto, H. Sugiyama, Y. Maeda, R. Sato, T., Tanaka, T. Matsunaga "Marine diatom, Navicula sp. strain JPCC DA0580 and marine Green Alga, *Chlorella* sp. strain NKG400014 as potential sources for biodiesel production," Appl. Biochemical Biotechnology., Humana Press, August **2009.**
[5] D. Singh, S. Singh, "Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: review," *Renewable and Sustainable Energy Reviews*, **2009**, 200-213.
[6] N. Mulumba, "Production of Biodiesel from Microalgae," M.S. Thesis, Chemical Engineering Dept., Univ. of New Hampshire, **2010**.
[7] R. Halim, M.K. Danquah, P.A. Webley, "Extraction of oil from microalgae for biodiesel production: A review", *Biotechnology Advances*, **2012**, 30, 3, 709-732.
[8] B. McConnell, I. Farag "Kinetics Study of the Solvent Extraction of Lipids from *Chlorella vulgaris*", *International Journal of Engineering and Technical Research*, **2013**, 1, 10, 28-37.
[9] L.M. Brown, S. Sprague, E. Jarvis, T. Dunahay, P. Roessler, K.G. Zeiler. Biodiesel from aquatic species. Project report: FY 1993, NREL/ TP-422-5726, 2-5, National Renewable Energy Lab, NREL: 1994.
[10] A. Demirbas, "Progress and recent trends in biodiesel fuels," *Energy Conversion and Management* **2009**, 50, 14–34.
[11] R. Wilson, I. Farag, "Parametric Study of Biodiesel Quality and Yield Using a Bench-Top Processor," *International Journal of Oil, Gas and Coal Technology* **2012**, 5, 1, 92 – 105.
[12] R. Wilson, G. Salama, I. Farag, "Microalgae Growth in Qatar for CO2 Capture and Biodiesel Feedstock Production," *Global Journal of Researches in Engineering, Chemical Engineering,* **2012**, 12, 1-9.
[13] S. Tewfik, S. Hawash, N. Atteya, G. El Diwani, I. Farag "Techno-Economic Appraisal of Biodiesel from Jatropha Curcas: An Egyptian case study," *Agricultural Science and Technology* B **2012**, 2, 287-297.
[14] K. Cui, "Gas Chromatograph Analysis of Biodiesel from Microalgae and Automation of Biodiesel Fuel Processor". B.S. Honors Thesis, Chemical Engineering Dept., University of New Hampshire, **2007**.
[15] J.M. Ferrentino, "Microalgae oil extraction and in situ transesterification," M.S. Thesis, Chemical Engineering Dept., University of New Hampshire, **2007**.
[16] N. Mulumba, I. Farag," Tubular Photobioreactor for Microalgae Biodiesel Production," *International Journal of Engineering Science and Technology*, **2012**, 4, 2, 703-709.
[17] T. Webster, "Temperature Stressing (Lipid Triggering) of Salt Water Algae for Enhanced Lipid Production," B.S. Honors Thesis, Chemical Engineering Dept., University of New Hampshire, **2009**.
[18] M. Elmoraghy, T. Webster, I. Farag "Microalgae Lipid Triggering by Cooling Stressing," *Journal of Energy and Power Engineering*, 2012, 6, 12, (Serial Number 61), 1918-1924.
[19] Z. Zuka, B. McConnell, I. Farag, "Comparison of Freshwater and Wastewater Medium for Microalgae Growth and Oil Production," *Journal of American Science* **2012**; 8(2):392-398.
[20] G. Chaput, K. Charmanski, I. Farag, "Sustainable Production of Microalgae Oil Feedstock Using Municipal Wastewater and CO2 Fertilization", *International Journal of Engineering Science and Technology*, **2012**, 4, 7, 3489-3499.
[21] K. Price, I. Farag, "Resources Conservation in Microalgae Biodiesel Production," *International Journal of Engineering and Technical Research*, **2013**, (October), 1, 8, 49-56.
[22] M. Elmoraghy, "Production of Bio-Jet Fuel from Microalgae," M.S. Thesis, Chemical Engineering Dept., Univ. of New Hampshire, **2013**.
[23] M. Elmoraghy, I. Farag, "Bio-jet Fuel from Microalgae: Reducing Water and Energy Requirements for Algae Growth," *International Journal of Engineering and Sciences, IJES*, **2012**, (Sept), l, 2, 22-30.
[24] M. Elmoraghy, I. Farag, "Wastewater and LED Microalgae Oil Production for Bio-Jet Fuel Manufacture," *Open Journal of Renewable and Sustainable Energy, RSE* **2014**, 1(2):11-20.
[25] B. McConnell, I. Farag, "Offshore Microalgae Growth in Wastewater," *Int. Review of Chemical Engineering*, **2012**, 4, 4, 423-429, July**.**
[26] B. McConnell, "Kinetics of microalgae oil extraction," B.S. Honors Thesis, Chemical Engineering Dept., Univ. of New Hampshire, **2013**.
[27] M. Laurens, M. Quinn, W. Stefanie, T. David, W. Edward, "Accurate and reliable quantification of total microalgal fuel potential as fatty acid methyl esters by in situ transesterification", An*al Bioanal Chem*, **2012**, 403, 167-178.
[28] K.G. Georgogiannia, M.G. Kontominasa, P.J. Pomonisa, D. Avlonitisb, V. Gergisc, "Conventional and in situ transesterification

of sunflower seed oil for the production of biodiesel". *Fuel Process. Technol*. **2008**, 89, 503–509.

[29] J. Qian, F. Wang, S. Liu, Z. Yun, "In situ alkaline transesterification of cottonseed oil for production of biodiesel and nontoxic cottonseed meal", *Bioresour. Technol.*. **2012**, 99, 9009–9012.

[30] L. Ramirez-Verduzco, J. Esteban Rodriguez-Rodriguez, A. del Rayo Jaramillo-Jacob, "Predicting Cetane number, kinematic viscosity, density and higher heating value of biodiesel from its fatty acid methyl ester composition", *Fuel*, **2012,** 91, 102-111.

Marian Elmoraghy graduated from the University of New Hampshire in September 2013 with an M.S. in Chemical Engineering. She completed several projects during her 4 years with the UNH Biodiesel Research Group. She has co-authored three peer-reviewed journal articles and given presentations at three professional and two graduate research conferences. She received an NSF Travel Grant to participate in the 2012 American Chemical Society (ACS) Green Chemistry Institute's Annual Green Chemistry & Engineering Conference.

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

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