CHARACTERIZATION OF MICROALGAE FOR THE PURPOSE OF BIOFUEL PRODUCTION

Z. Bi, B. B. He

ABSTRACT. It is now widely accepted that microalgae are promising candidate feedstocks for biofuel production, especially for biodiesel. Microalgae consist of a vast number of species that contain complex chemical constituents and physical structures. The purpose of this study is to understand the physical and chemical properties of selected microalgae, which is critical to the design of appropriate processes for commercial biofuel production. ASTM standard methods were implemented to examine the microalgae properties, including proximate and ultimate analyses. Among the microalgae studied, green microalgae have more volatile matter than brown microalgae, while the latter contain much higher ash content (as high as 43.4%wt ± 0.20%wt dry basis). The lowest ash content was found in the samples of green microalgae (14.3%wt ± 0.10%wt dry basis). Ultimate analysis showed that brown microalgae have less carbon content (approx. 25%wt dry basis) as compared to green microalgae (49%wt to 58%wt dry basis). All samples of microalgae were high in sulfur content (0.4%wt to 1.0%wt dry basis). Mineral contents of all microalgal samples were similar to those commonly present in other biomass. Brown microalgae contain significantly higher amounts of carbohydrates (72.9%wt to 75.5%wt dry basis) than green microalgae. On the other hand, green microalgae contain more crude fat (17.1%wt to 27.8%wt dry basis) than brown microalgae. The fatty acid profiles show that the primary fatty acids in microalgal lipids are similar to those of vegetable oils such as soybean oil. However, there are also many odd-numbered fatty acids, such as C15:0, C17:0, and C19:0, which are not typically seen in other seed oils.

Keywords. Biodiesel, Biofuels, Biomass, Characterization, Fatty acids, Microalgae.

Biomass is a viable renewable energy feedstock that promotes production of sustainable energy and reduction in greenhouse gas emissions (Kröger and Müller-Langer, 2012). Among the various types of biomass, microalgae are promising candidates because of their high biomass yields, high lipid contents, low cultivation costs, and non-competition with agricultural land. In addition, microalgae fix CO₂, thereby reducing net carbon addition to the atmosphere. These benefits give microalgae unique status as an environmentally friendly resource for large-scale production of biofuels. Microalgae are unicellular microscopic plants, heterotrophic or autotrophic photosynthesizing organisms that grow in freshwater and marine systems (Chisti, 2010; Tang et al., 2010). As some microalgae are not suitable for biofuel production, understanding the physical and chemical properties of microalgae is critical to developing appropriate processes for commercial biofuel production. This study aims to characterize the chemical, biochemical, and physical properties of selected microalgae species that hold potential for use as biofuel feedstocks.

Researchers have explored microalgae from various aspects, and proximate and ultimate analyses have been the most commonly conducted tests (Ross et al., 2008; Naik et al., 2010; Phukan et al., 2011; Wu et al., 2012; Rizzo et al., 2013). Proximate analysis has been the most commonly used method for characterizing coal and other energy fuel sources (Stahl et al., 2003; Ucar and Ozcan, 2008; García et al., 2012). The American Society for Testing and Materials (ASTM) developed a series of standards for this analysis (ASTM, 2006). Ultimate analysis, also known as the element test, is the determination of carbon and hydrogen, as well as sulfur, nitrogen, and oxygen, in a material through their gaseous products in complete combustion. Both proximate and ultimate analyses can be done according to ASTM Standard E870-82 (ASTM, 2006). Determination of the elemental composition of whole biomass, including various minerals in the ash, is also helpful in understanding microalgae as a feedstock for biofuel production (Borah et al., 2009).

Thermogravimetric analysis (TGA) is an emerging analytical technique that has been used to determine proximate biochemical compositions. TGA is based on continuous measurement of biomass weight loss versus temperature increase. Weight loss may arise from the evaporation of residual moisture or solvent, or from the decomposition of organic matter at higher temperatures. If oxygen or air is used as the carrier gas, then oxidation of organic matter...
also contributes to the weight loss (Tang et al., 2011; Chen et al., 2011). Although TGA is useful in determining volatile matter, its accuracy remains approximate because TGA cannot precisely distinguish the components within a mixture. Nevertheless, TGA is still a widely used technique for characterizing biomass. For instance, using TGA, Phukan et al. (2011) conducted thermal analysis on the microalgae *Chlorella* sp. to determine the lipid, energy, and ash contents. Moreover, Griersson et al. (2009) characterized six microalgae species under slow pyrolysis conditions and reported the production yields, compositions, and calorific values of lipids, gas, and char at 500°C. A variation of the TGA method is to record the weight loss over time at a constant temperature, or isothermal TGA (Zou et al., 2010; Peng et al., 2001; Chen et al., 2011). Some instruments are designed to record and process the data of differential scanning calorimeters (DSC) and TGA simultaneously (Come-saña et al., 2012).

According to the integrated report on renewable fuels by Stahl et al. (2003), TGA can provide a proximate analysis. In the research conducted by Silva et al. (2008), loss of moisture or free water began at approximately 108°C, followed by mass losses in two stages at approximately 240°C and 580°C in an oxygen environment. Marcilla et al. (2009) obtained similar results, with three stages of decomposition using TGA. In TGA with nitrogen as the carrier gas, the first-stage mass loss (up to 110°C) generally refers to the moisture content, and the following mass loss (between 110°C and 900°C) is the volatile matter. Upon reaching 900°C, samples are completely volatilized, and the leftover mass is considered a combination of fixed carbon and ash, which are difficult to quantify individually with TGA alone. Silva et al. (2008) claimed that after water was released at about 108°C, the marine macroalgae *Ulva lactuca* deformed dramatically in two stages, at approximately 240°C and 580°C, until the formation of ash at nearly 650°C. These researchers explained that the sample decomposition started at 240°C and 580°C, and organic compounds volatilized in the presence of oxygen. Hence, the mass difference between 240°C and 580°C was considered the combined weight loss of both volatile matter and fixed carbon content.

The fatty acid profile is important in selecting microalgae as a feedstock for biofuel production. The most common approach to determining the fatty acid profiles of microalgal oils is to extract the lipids through a liquid-liquid extraction (or solvent extraction) and then analyze them using gas chromatography (Medina et al., 1998). At laboratory scale, Soxhlet extraction and modified Bligh-Dyer extraction are commonly used methods. Soxhlet extraction is often performed in a hot mode, and a non-polar organic solvent, such as hexane, is typically used (Cooney et al., 2011; Smedes and Thomasen, 1996; Mani et al., 2004; Lewis et al., 2000). The Bligh-Dyer method is recommended for extracting neutral lipids (i.e., triglycerides, waxes, and pigments) and cell membrane lipids (i.e., phospholipids, glycoproteins, and cholesterol) due to its combined polar solvents, chloroform and methanol (Manirakiza et al., 2001; Ferraz et al., 2004). Soxhlet extraction has been found unsuitable for water-rich samples, while Bligh-Dyer extraction gives good extraction efficiency for samples with high water content since it can use both polar and non-polar solvents. Therefore, Bligh-Dyer extraction can extract polar and non-polar lipids with polar solvents, especially from samples with high water content, because Bligh-Dyer extraction uses chloroform or a mixture of methanol and chloroform to extract neutral lipids, and methanol as the solvent to extract polar lipids in cell membranes (Ferraz et al., 2004). On the other hand, Soxhlet extraction uses hexane and can only extract neutral lipids (Ferraz et al., 2004).

In analyzing the lipids extracted from various microalgae samples, GC methods give fairly reliable fatty acid profiles for the purpose of biodiesel production (Bigogno et al., 2002; Petkov and García, 2007; Damiani et al., 2010; Gressler et al., 2010; Halim et al., 2011).

In examining the physical structures of microalgae, scanning electron microscopy (SEM) provides good visual images of possible microalgal cell structure and/or morphology changes after treatment, such as drying and solvent extraction, and indirectly provides information about the solvent extraction efficiency, as it is used in examining coal surface structure after complete combustion (Suárez-García et al., 2002). SEM scans solid samples with a beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample to produce signals that contain information about the sample’s surface topography, composition, and other properties, such as electrical conductivity (Cooney et al., 2009; Reimer, 1985).

Microalgae are traditionally considered a valuable nutrient source for use as health supplements. Current markets for microalgae are largely in the sectors of human health food and animal feed due to the high concentrations of long-chain unsaturated fatty acids, such as omega-3 and omega-6 (Hammond et al., 2001; Adarme-Vega et al., 2012). The high concentrations of proteins and carbohydrates make microalgae an ideal source of nutrients for preparing functional foods, food additives, and even nutraceuticals. Therefore, determining the carbohydrate, protein, and crude fat contents of microalgae will provide additional information from another point of view. The lignocellulose in microalgae could be a raw material for producing bioethanol that can be considered as the derivative carbohydrates (Hu et al., 2011).

**MATERIALS AND METHODOLOGY**

**SAMPLES OF MICROALGAE**

Samples from two microalgae genera (green and brown microalgae) were de-watered by different methods and tested in this study. Five samples were green microalgae, including: freeze-dried (FD) mixed marine green microalgae (Mixed Green I), spray-dried (SD) mixed marine green microalgae (Mixed Green II), refractance window-dried (RW) MCD#TD 1440, freeze-dried *N. salina* (FD *N. salina*), and oven-dried *N. salina* (OD *N. salina*). Three samples were brown microalgae, including: freeze-dried mixed marine brown microalgae (Mixed Brown I), spray-dried
mixed marine brown microalgae (Mixed Brown II), and refractance window-dried (RW) MCD##TD 1427. These microalgae samples were purchased separately from commercial companies: Mixed Green I and Mixed Green II from Algal Technology (Cumming, Ga.), Mixed Brown I and Mixed Brown II from Algae Bioscience (Scottsdale, Ariz.), and MCD##TD 1427 and 1440 from MCD Technologies, Inc. (Tacoma, Wash.). Samples of OD N. salina, cultured by Solix BioSystems, Inc. (Fort Collins, Colo.), were prepared by low-temperature drying at 60°C for 72 h according to ASABE Standard S358.2 (ASABE Standards, 1998). Samples of FD N. salina were prepared by low-temperature drying in a freeze dry system (FreeZone 4.5, Labconco, Kansas City, Mo.) under 0.133 mbar vacuum and -50°C for at least 72 h.

All reagents used in this research were HPLC-grade chemicals. The n-hexane solvent for Soxhlet extraction was purchased from Fisher Scientific (Pittsburgh, Pa.). The chloroform and methanol used in modified Bligh-Dyer extraction were products of EMD (Darmstadt, Germany) and obtained from the Chemical Store at the University of Idaho.

PROXIMATE ANALYSIS
A model Q50 TGA (TA Instruments, New Castle, Del.) was used for the TGA tests. A pair of platinum weight pans (100 µg) was used for containing the samples. Nitrogen was used as the carrier gas to protect the samples from oxidation. Microalgae samples were heated to 900°C at a rate of 5°C min⁻¹, and resulting volatile products were analyzed with Thermal Analysis Universal software (TA Instruments, New Castle, Del.).

In addition to the TGA tests, Karl-Fischer titration, using a DL38 titrator (Mettler-Toledo, Columbus, Ohio), was also performed for moisture determination. Dry oxidation in a hot-air furnace (Thermo Scientific, Asheville, N.C.) was applied to determine the ash content of microalgae samples following ASTM Standard D1102-84 (ASTM, 2007). The fixed carbon content was determined by subtracting the summation of the moisture content, volatile matter, and ash content from the total sample mass.

ULTIMATE ANALYSIS
The ultimate analysis for elemental content of all samples was conducted by two professional analytical laboratories. Carbon (C), nitrogen (N), and sulfur (S) were tested by the Analytical Sciences Laboratory of the University of Idaho. In determining C, S, and N, a complete combustion process converts the elemental carbon, sulfur, and nitrogen into CO₂, SO₂, N₂, and NO₃. These gases are then passed through infrared cells to determine the C and S contents, and through a thermal conductivity cell to determine the N content. The reporting limits are 0.01% to 0.02% for all three elements for a sample size of approximately 0.25 g.

Oxygen (O) content was analyzed by the Nutrition Analysis Center, Eurofins Scientific, Inc. (Des Moines, Iowa). A FlashEA 1112 elemental analyzer (Thermo Scientific, Asheville, N.C.) was used to pyrolyze the sample (1.0 to 4.0 mg in silver weighing capsules) in an inert atmosphere (helium) at 1060°C to produce CO, which was detected automatically in a self-integrating, steady-state thermal conductivity analyzer (Galbraith Laboratories, Inc., Knoxville, Tenn.). A microprocessor calculates the oxygen content as weight percentage based on manual entries.

Mineral determination was implemented by the Analytical Sciences Laboratory at the University of Idaho. A sample size of 1.0 g wet weight or 0.25 g dry weight was required for this procedure. Samples were digested in 30% nitric acid, and minerals were determined with an inductively coupled plasma atomic emission spectrometer (ICP-AES, Optima 3200RL, Perkin Elmer, Waltham, Mass.).

MODIFIED BLIGH-DYER EXTRACTION
In Bligh-Dyer extraction, the extraction mixture was prepared in a beaker at room temperature according to the following procedure (Lewis et al., 2000) with modified reagent dosage. A 20 mL volume of methanol (CH₃OH) and 10 mL of chloroform (CHCl₃) were mixed with 5 g of dried microalgae sample by vortexing at low speed for 5 min. Then 18 mL of deionized water was added to the mixture, which was vortexed for another 2 min. An additional 10 mL of chloroform was added and vortexed for 5 min to ensure complete extraction. After extraction, the water, solid biomass, and biphasic miscella were separated by centrifugation. Water migrated to the top, solid biomass to the middle layer, and biphasic miscella to the bottom of the centrifuge tube. The water was then carefully removed by pipette, and the biomass and miscella were separated by filter paper. Finally, the methanol and chloroform were evaporated out of the miscella with a vacuum evaporator (Rotavapor R-14, Buchi AG, Flawil, Switzerland) at 104°C and 500 mbar for 30 min.

SAXHLET EXTRACTION
Soxhlet extraction was performed with a Soxhlet system manufactured by Synthware (Beijing, China). In Soxhlet extraction, 150 mL of n-hexane as the extract solvent was added to the Soxhlet device, which was operated for 3 to 4 h to achieve maximum extraction efficiency. After extraction, the same vacuum evaporator as used in the modified Bligh-Dyer extraction was used to evaporate the n-hexane at 35°C and 500 mbar for 30 min.

GAS CHROMATOGRAPHY
Lipid samples extracted from microalgae by the aforementioned methods were analyzed for their fatty acid profiles by GC. A 1 µL sample was injected into a gas chromatograph (5890 Series II, Hewlett-Packard, Palo Alto, Cal.) equipped with a flame ionization detector at 300°C and a J&W 30 m × 0.25 mm i.d. DB-23 column (0.25 µm film thicknesses). The split injection port was set to achieve a 100:1 ratio, and the inlet port temperature was maintained at 250°C. An initial column oven temperature of 215°C was held for 3 min and then increased to 230°C at a rate of 3°C min⁻¹. The carrier gas was helium at 1 mL min⁻¹, and nitrogen makeup gas was supplied to the detector at 30 mL min⁻¹. The weight percentages of the fatty acid methyl esters were quantified with a Hewlett-Packard 3396 Series II integrator.
SCANNING ELECTRON MICROSCOPY IMAGES

SEM images were taken for analyzing cell structure changes of the microalgae samples. An imaging SEM (200F, FEI Co., Hillsboro, Ore.) at the Franceschi Microscopy and Imaging Center, Washington State University, was employed to take images of microalgae samples at three different magnifications: 5,000×, 10,000×, and 15,000×.

CARBOHYDRATES, PROTEIN, AND CRUDE FAT

The crude fat contents of the microalgae samples were tested by the Nutrition Analysis Center, Eurofins Scientific, Inc. (Des Moines, Iowa). Acid hydrolysis was used, following AOAC Standard 954.02 (AOAC, 2005), to determine the crude fat content. The protein contents were converted from the nitrogen content in ultimate analysis. The conversion factor (×6.25) was referenced according to the Kjeldahl method (Wrolstad et al., 2005). The carbohydrate contents were determined by subtracting the ash content, crude fat content, and protein content from 100%.

RESULTS AND DISCUSSION

Figure 1. Example TGA profiles of two microalgal samples.
TGA is a powerful technique that can provide thermal property information for each microalgal sample. Figure 1 shows example TGA profiles of two microalgae samples. According to the experiment conducted by Silva et al. (2008), free water or moisture content (MC) are released from biomass starting at around 110°C to approximately 200°C, as indicated by the first significant peak on the derived weight change curve (DWCC) in our study.

TGA is capable of determining MC, volatile matter (VM), fixed carbon (FC), and ash content. According to the literature (Silva et al., 2008; Marcilla et al., 2009; Zou et al., 2010; Chen et al., 2011), volatile matter provides the components lost between 160°C and 700°C, and the ash content is the mass left after 900°C. The fixed carbon is determined by the difference between the total sample mass and the contents of moisture, volatile matter, and ash. Therefore, in proximate analysis by TGA, the mass loss before 110°C refers to MC, and the mass loss between 110°C to 900°C is summarized as VM. The remaining mass includes both FC and ash. The TGA results are summarized in table 1.

As shown in figure 1, after moisture loss at around 110°C, two more stages of mass loss, at approximately 240°C and 580°C, follow due to the loss of volatile matter and the decomposition of lignocellulosic polymers (Silva et al., 2008; Marcilla et al., 2009; Zou et al., 2010; Chen et al., 2011). After 200°C, the TGA profiles show that all microalgal species have one or two significant peaks in the vicinity of 300°C, which are believed to be the loss of lipids and some decomposition of lignocellulosic polymers. However, neither the total lipid content nor the lipid composition can be distinguished due to the sensitivity and selectivity limitations of TGA, unless a mass spectrometry (MS) analyzer is connected to the TGA analyzer to further determine individual components (Ioannidou et al., 2011).

Generally speaking, thermal processes (e.g., liquefaction and gasification) require a certain level of moisture content in the feedstock to achieve an acceptable thermal conversion efficiency. Large-scale processing usually requires 8%wt to 10%wt moisture in the feedstock (Brown, 2003). Too much moisture interferes with the conversion, and drying is the most costly stage in the whole biomass processing procedure (Brown, 2003). The TGA profiles provided substantial evidence of microalgae MC. Karl-Fischer analyses were also implemented to confirm the TGA data. Table 1 illustrates that green microalgae have relatively less moisture (average of 4.7%wt) than brown microalgae (average of 5.8%wt) under the same drying conditions and thus are assumed to be more suitable for direct thermal processing without the added cost of moisture removal.

Comparison of the TGA profiles of green and brown microalgae shows a significant difference: green microalgae had the major VM loss at approximately 280°C, while brown microalgae had the major VM loss close to 900°C. This is an unusual thermal behavior in biomass thermal testing due to the fact that cellulose decomposes between 290°C and 390°C, lignin decomposes between 200°C and 700°C (Sanchez-Silva et al., 2012), and most organic materials decompose between 160°C and 555°C (Peng et al., 2001; Marcilla et al., 2009; Zou et al., 2010; Chen et al., 2011; Chen et al., 2012). The distinctive VM loss of brown microalgae at this high temperature might be due to the higher lignin content in brown microalgae, as lignin is very difficult to decompose. Some studies defined TGA of thermally decomposed biomass with pure nitrogen as pyrolysis, and this was reported to be frequently used in microalgae characterization (Wu et al., 2012; Sanchez-Silva et al., 2013; Maddi et al., 2011). In those studies, considering the thermal degradation properties of green microalgae, the char (ash) content was around 20%. Maddi et al. (2011) stated that hemicellulose is more thermally labile than cellulose and decomposes at 220°C to 315°C in an oxygen-free atmosphere, followed by cellulose at 315°C to 400°C. In contrast, lignin is a relatively more heterogeneous polymer than hemicellulose and cellulose and hence decomposes over a much wider temperature range (190°C to 900°C) (Maddi et al., 2011), which could explain the significant peak on the mixed brown microalgae TGA derivative weight loss curve at around 900°C.

A furnace method, following ASTM Standard D1102-84 (ASTM, 2007), was employed to determine the ash content to complete the proximate analysis. The data in table 2 show that green microalgae contain much less ash than brown microalgae. Mixed Brown II has a surprisingly high ash content of 43%wt, which would be a negative factor in processing it for biofuels. Additionally, the ash content measured by the furnace method, which heated microalgae to 600°C in air, is significantly higher than the combined FC and ash determined by TGA. All three brown microalgae samples distinguishably have much more residue (approx. 25%wt wet basis) at 900°C. In recent years, many researchers have worked on discovering the characteristics of various microalgae. Most of their studies used green microalgae, and the results showed that about 20%wt of the microalgal biomass was left after slow pyrolysis and became char (Wu et al., 2012; Sanchez-Silva et al., 2013).
Oxidation of the incorporated minerals in microalgae into mineral oxides in the open-to-air furnace is the most likely contributor to this significant difference.

Ultimate analysis is the elemental determination of carbon, hydrogen, oxygen, nitrogen, and sulfur (ASTM, 2006). It is essential to perform an ultimate analysis on microalgae to fully understand the beneficial effects of using the microalgae as a biofuel feedstock before further processing (Suárez-García et al., 2002). Moreover, the elemental contents of carbon, hydrogen, and oxygen in microalgae can approximately correlate to the gross calorific value of the microalgae biofuel. Table 3 summarizes the results of ultimate analysis on the selected microalgal samples. Carbon, hydrogen, oxygen, nitrogen, and sulfur are the major elements in microorganisms, up to 96% on dry weight basis. Due to facility limitations, hydrogen content could not be determined directly. Based on the work by Phukan et al. (2011), the green microalgae *Chlorella* consists of 47.54% carbon, 7.1% hydrogen, 38.6% oxygen, and 6.73% nitrogen, with a gross calorific value of 18.59 MJ kg\(^{-1}\). *Chlorella* is a genus of green microalgae that is similar to the microalgae used in this study. Therefore, the hydrogen content of the microalgal samples tested in this study was assumed to be in a similar range, i.e., approximately 7%wt.

It is known that the carbon content of the biomass correlates proportionally with the heating value of the biofuel, while the oxygen content affects the heating value in an inverse manner. High oxygen content is not a desired property for high energy density biofuels. Results from this study showed that green microalgae contain higher carbon content than brown microalgae. Therefore, green microalgae are assumed to be better candidates for biofuel production in terms of energy content. Meanwhile, low nitrogen and low sulfur content in microalgal biomass are highly desirable for making environmental friendly biofuels. Results showed that the microalgal biomass samples tested in this study all contain very high levels of nitrogen and sulfur. Although the sulfur content of the green microalgae is lower than that of the brown microalgae, both are high in sulfur and nitrogen as compared to other types of biomass, such as oat straw, which contains 1.06% nitrogen and less than 0.2% sulfur (Ross et al., 2008). Thus, processing of the microalgal biomass would require additional treatments to remove the undesirable heterogeneous elements in order to meet the biofuel specifications.

Ash is a byproduct of most biomass thermal conversion processes, including pyrolysis and gasification. Ash content would very likely affect the process design and operation, as well as the product purification processes and product quality. Examining individual minerals in the microalgae biomass also provides valuable information for the microalgae cultivation from the aspect of nutrient requirements. Naturally, the ash from biomass combustion contains high levels of nutrient elements from fertilizer and substrates, such as sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), and phosphorus (P), as well as potentially toxic metals (Wang et al., 2012). Among other mineral elements, arsenic (As), chromium (Cr), cobalt (Co), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) are the common minor and trace elements that occur in plants at various concentrations. Compared to oat straw, miscanthus, and willow coppice, microalgae contain relatively high levels of macro-minerals (Na, K, Ca, P, and Mg) and trace elements (Al, Cu, Fe, Zn, and Mn) (Ross et al., 2008). Knowing the mineral content is important for designing conversion processes because of their impact on slagging, fouling, and other ash-related problems. Experi-

### Table 2. Ash contents of eight microalgal samples by furnace determination.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Microalgae Species</th>
<th>Ash Content (%wt dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FD Mixed Green I</td>
<td>17.3 ±0.14</td>
</tr>
<tr>
<td>2</td>
<td>SD Mixed Green II</td>
<td>14.3 ±0.10</td>
</tr>
<tr>
<td>3</td>
<td>RW MCD#TD 1440 (green)</td>
<td>16.4 ±0.12</td>
</tr>
<tr>
<td>4</td>
<td>FD <em>N. salina</em> (green)</td>
<td>13.8 ±0.09</td>
</tr>
<tr>
<td>5</td>
<td>OD <em>N. salina</em> (green)</td>
<td>18.8 ±0.18</td>
</tr>
<tr>
<td>6</td>
<td>FD Mixed Brown I</td>
<td>28.1 ±0.17</td>
</tr>
<tr>
<td>7</td>
<td>SD Mixed Brown II</td>
<td>43.4 ±0.20</td>
</tr>
<tr>
<td>8</td>
<td>RW MCD#TD 1427 (brown)</td>
<td>41.6 ±0.05</td>
</tr>
</tbody>
</table>

### Table 3. Ultimate analysis of microalgal samples.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Microalgae Species</th>
<th>Elemental Composition (%wt dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Carbon</td>
</tr>
<tr>
<td>1</td>
<td>FD Mixed Green I</td>
<td>58</td>
</tr>
<tr>
<td>2</td>
<td>SD Mixed Green II</td>
<td>58</td>
</tr>
<tr>
<td>3</td>
<td>RW MCD#TD 1440 (green)</td>
<td>56</td>
</tr>
<tr>
<td>4</td>
<td>FD <em>N. salina</em> (green)</td>
<td>51</td>
</tr>
<tr>
<td>5</td>
<td>OD <em>N. salina</em> (green)</td>
<td>49</td>
</tr>
<tr>
<td>6</td>
<td>FD Mixed Brown I</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
<td>SD Mixed Brown II</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>RW MCD#TD 1427 (brown)</td>
<td>24</td>
</tr>
</tbody>
</table>

### Table 4. Mineral contents in ashes of eight different microalgal samples.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Microalgae Species</th>
<th>Mineral Contents (μg g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>As</td>
</tr>
<tr>
<td>1</td>
<td>FD Mixed Green I</td>
<td>&lt;16</td>
</tr>
<tr>
<td>2</td>
<td>SD Mixed Green II</td>
<td>6.5</td>
</tr>
<tr>
<td>3</td>
<td>RW MCD#TD 1440 (green)</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>FD <em>N. salina</em> (green)</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>OD <em>N. salina</em> (green)</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>FD Mixed Brown I</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>SD Mixed Brown II</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>RW MCD#TD 1427 (brown)</td>
<td>16</td>
</tr>
</tbody>
</table>
mental results in this study indicate that microalgae have surprisingly high metal levels (table 4).

Testing for carbohydrates, protein, and crude fat is necessary for understanding microalgae from another point of view. All three brown microalgae have consistently higher carbohydrate contents than green microalgae, while green microalgae contain high crude fat (table 5). Therefore, brown microalgae may have the potential for bioethanol production by utilizing their high carbohydrate content, while green microalgae could be used for biodiesel production by taking advantage of their higher fat content. High protein content implies high nitrogen content, which, as discussed previously, is undesirable for biofuel production.

To take advantage of the high fat content of green microalgae for biodiesel production, the traditional method is to remove the lipids from the microalgal biomass by solvent extraction before further processing. Solvent extraction is a physical process that may affect the physical structure of the microalgae. The physical structure changes before and after solvent extraction or among different drying processes, providing a visual indication on the effectiveness of the solvent extraction or drying process. In this study, SEM images were taken of microalgae samples, and physical structure changes were analyzed.

Figure 2 shows examples of SEM visualization of two microalgae. The SEM images show that solvent extraction does not significantly change the cell morphology, but the drying process does. The images were taken at 15,000× magnification on green microalgal biomass from different drying methods and before and after Soxhlet extraction. The SEM images show that there is no significant morphology change after solvent extraction. The SEM images of Mixed Green II show that the cell surfaces remain intact with slightly wider pore size after extraction. The images of Mixed Green I show that the cell structure had been totally destroyed, as no morphology change was demonstrated. The same phenomenon occurred with microalgae treated with modified Bligh-Dyer extraction. This suggests that Soxhlet and Bligh-Dyer extractions do not significantly impact the cell structure. However, the microalgal cells after different drying processes show considerably different structures. For example, the images show that the freeze-dried cells of Mixed Green I collapse into pieces, while the spray-dried cells have an intact structure. Therefore, the lipids in Mixed Green II penetrated the cell wall via cell pores, but the lipids in Mixed Green I diffused into the chemicals and were extracted. Cooney et al. (2011) compared the lipid extraction yields from dried and undried microalgae, and their results suggested that the drying process mechanically breaks down the cell wall, causing higher extraction yields. Their results also suggested that the presence of water has the effect of blocking solvent access (Cooney et al., 2011). Although the SEM images did not show the effects of solvent extraction, the fatty acid profile illustrates that lipids were extracted. Therefore, the microalgae after solvent extraction may have had some structural changes that were not visually significant. It could be assumed that solvent extraction causes more hollow structures in microalgae.

Knowledge of the lipid profile can offer significant information for biofuel research, such as the selection of microalgal strains and optimization of microalgae growth and extraction conditions. The lipid profile is one of the most important characteristics in making biodiesel. Typically, characterization of microalgal lipids involves breaking down the triglycerides and other intact lipids, followed by the derivation of fatty acids to fatty acid methyl esters prior to analysis by GC. In this study, the fatty acid profiles were analyzed by GC on 13 solvent-extracted lipid samples. The results are organized based on the signatures of carbon chain length and saturation of chemical bonds (tables 6 and 7).

Soxhlet and modified Bligh-Dyer solvent extractions were performed on the eight available microalgae samples. Six samples, i.e., Mixed Green I, Mixed Green II, RW MCD#TD 1440, RW MCD#TD 1427, FD N. salina, and OD N. salina, successfully provided quantifiable lipids by Soxhlet extraction, and six other samples, i.e., Mixed Green I, Mixed Green II, Mixed Brown II, RW MCD#TD 1427, FD N. salina, and OD N. salina, successfully provided quantifiable lipids by Bligh-Dyer extraction. Therefore, the results in tables 6 and 7 indicate that extraction of microalgal lipids may depend on the characteristics of the individual microalgal strains. The results also show that the fatty acid profiles of lipids from the same microalgae vary considerably between the two extraction methods, as discussed below.

Soxhlet extraction worked on every green microalga and some of the brown microalgae, while Bligh-Dyer extraction worked better on brown microalgae. Bligh-Dyer extraction appears to be more effective in extracting microalgal lipids than Soxhlet extraction. This was evidenced by the constantly higher total lipid yields from Bligh-Dyer extraction than from Soxhlet extraction, although both extraction methods achieved similar yields with FD N. salina and OD
Meanwhile, most of the fatty acids in microalgal lipids are saturated fatty acids and relatively long-chain molecules of C14 to C18 triglycerides, which is the preferred range for biodiesel. The data in tables 6 and 7 show that saturated fatty acids are the major components in microalgal lipids (30% to 60% of the total). Among the saturated fatty acids, C16:0 (palmitic acid) is the major portion, which is similar to many other vegetable oils, such as soybean oil. In a typical soybean oil, 6% to 8% of the lipids are palmitic acid (C16:0), 2% to 5% are stearic acid (C18:0), 20% to 30% are oleic acid with one double bond (C18:1), 50% to 60% are linoleic acid with two double bonds (C18:2), and 5% to 11% are linolenic acid with three double bonds (C18:3) (Van Gerpen and He, 2010). These lipids were found in the microalgal oils in relatively similar proportions. The oils of Mixed Green II and RW MCD#TD 1427 are exceptions in which linolenic acid (C18:3) is missing (tables 6 and 7). This finding indicates that microalgal oils are good candidates for biodiesel production because their fatty acid profiles are similar to that of soybean oil, which is currently the primary resource of biodiesel production in the U.S.

The fatty acid profiles obtained from the selected microalgal oils in this study are also in agreement with those published for various strains by other researchers. The tested microalgal oils all contain C16:0, C18:1, and C18:2 fatty acids, which are the major constituents of their fatty acid profiles (Ehimen and Carrington, 2010; Demirbaş, 2009; Damiani et al., 2010; Halim et al., 2011; Liu et al., 2011).
Table 6. Fatty acids in microalgal lipid samples in this study by Soxhlet extraction.[a]

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Microalgae Species</th>
<th>Saturated Fatty Acids (%wt)</th>
<th>Monounsaturated Fatty Acids (%wt)</th>
<th>Polyunsaturated Fatty Acids (%wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FD Mixed Green I</td>
<td>2.7 2.8 2.9 2.9 18.2 1.0 6.6 0.5 0.5 1.3 39.4</td>
<td>0.5 0.5 1.5 2.0 16.2 0.4 0.4 0.5 22.0</td>
<td>3.0 23.3 0.6 3.1 1.6 5.1 0.5 0.5 1.0 38.7</td>
</tr>
<tr>
<td>2</td>
<td>SD Mixed Green II</td>
<td>0.3 - 3.5 0.5 35.5 0.5 1.4 - 0.9 12.8 55.4</td>
<td>- - 14.6 - 3.9 - - - 18.5</td>
<td>- - 21.0 4.7 - - 16.1 60.2</td>
</tr>
<tr>
<td>3</td>
<td>RW MCD#TD 1427 (brown)</td>
<td>- - 0.8 - 30.0 0.8 1.8 - - 1.4 34.8</td>
<td>- - 16.7 - 4.1 - - - 20.7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>FD N. salina (green)</td>
<td>0.2 - 1.3 - 26.5 1.1 0.9 - 0.5 2.9 33.4</td>
<td>- - 3.8 11.6 - 32.9 0.7 - - 49.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>OD N. salina (green)</td>
<td>0.2 - 3.2 0.5 34.0 0.5 1.0 - 0.8 14.8 55.0</td>
<td>- - 16.7 - 4.1 - - - 20.7</td>
<td></td>
</tr>
</tbody>
</table>

[a] Systematic names of fatty acids:
C12:0 = dodecanoic, C13:0 = tridecanoic, C14:0 = tetradecanoic (myristic acid), C15:0 = pentadecanoic, C16:0 = hexadecanoic acid (palmitic acid), C17:0 = heptadecanoic (margaric acid), C18:0 = octadecanoic acid (stearic acid), C19:0 = nonadecanoic, C20:0 = eicosanoic (arachidic acid), C22:0 = docosanoic acid (behenic acid).

C14:1 = tetradecenoic acid (myristoleic acid), C16:1 = hexadecenoic acid (palmitoleic acid), C18:1 = octadecenoic acid (oleic acid), C20:1 = eicosenoic acid (gadoleic acid), C22:1 = docosenoic acid (erucic acid).

C18:2 = octadecadienoic acid (linoleic acid), C18:3 = octadecatrienoic acid (linolenic acid), C20:4 = eicosatetraenoic acid (arachidonic acid), C22:4 = docosadienoic acid (brassic acid).

[b] T = trans.
[c] TT = trans at both double bonds.
[d] Double bonds at positions 8, 11, and 14 are typically noted as 8-11-14.
[e] Double bonds at positions 11, 14, and 17 are typically noted as 11-14-17.

Table 7. Fatty acids in microalgal lipid samples in this study by modified Bligh-Dyer extraction.[a]

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Microalgae Species</th>
<th>Saturated Fatty Acids (%wt)</th>
<th>Monounsaturated Fatty Acids (%wt)</th>
<th>Polyunsaturated Fatty Acids (%wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FD Mixed Green I</td>
<td>0.2 3.3 0.4 38.6 0.6 1.0 - 16.1 60.2</td>
<td>0.1 - 21.0 4.7 - - - 25.8</td>
<td>3.0 0.6 2.8 - - - 9.0</td>
</tr>
<tr>
<td>2</td>
<td>SD Mixed Green II</td>
<td>- 3.6 0.4 32.3 - 0.9 - 21.8 59.0</td>
<td>- - 19.4 5.6 - - - 25.0</td>
<td>- - 15.5 4.9 - - - 20.7</td>
</tr>
<tr>
<td>3</td>
<td>RW MCD#TD 1427 (brown)</td>
<td>- 18.1 0.9 11.4 1.8 14.6 - 4.5 51.3</td>
<td>- 0.6 15.0 2.8 - - - 18.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>FD N. salina (green)</td>
<td>0.3 1.7 0.2 32.4 0.9 1.9 - 3.1 40.5</td>
<td>- 3.4 11.9 30.0 0.6 46.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>OD N. salina (green)</td>
<td>- 1.4 0.3 32.5 0.9 1.3 0.5 2.2 39.1</td>
<td>- - 16.4 27.2 0.5 44.2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>SD Mixed Brown II</td>
<td>- 13.0 0.8 10.7 2.6 0.5 0.2 9.7 37.5</td>
<td>- - 16.4 27.2 0.5 44.2</td>
<td></td>
</tr>
</tbody>
</table>

[a] Systematic names of fatty acids:
C12:0 = dodecanoic, C13:0 = tridecanoic, C14:0 = tetradecanoic (myristic acid), C15:0 = pentadecanoic, C16:0 = hexadecanoic acid (palmitic acid), C17:0 = heptadecanoic (margaric acid), C18:0 = octadecanoic acid (stearic acid), C19:0 = nonadecanoic, C20:0 = eicosanoic (arachidic acid), C22:0 = docosanoic acid (behenic acid).

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C18:2 = octadecadienoic acid (linoleic acid), C18:3 = octadecatrienoic acid (linolenic acid), C20:4 = eicosatetraenoic acid (arachidonic acid), C22:4 = docosadienoic acid (brassic acid).

[b] T = trans.
CONCLUSIONS

The goal of this research was to determine the physical and chemical profiles of selected microalgae in order to prove their worth as biofuel feedstocks. The results demonstrated that microalgae could be an alternative to other well-known biofuel feedstocks. TGA analysis showed that brown microalgae generally contain larger quantities of less volatile matter than green microalgae. FD N. salina contained the least amount of ash, and all of the brown microalgae strains contained much higher ash contents. Ultimate analysis indicated that both FD and SD mixed marine green microalgae are better options for producing a high heating value biofuel due to their higher carbon content and lower oxygen content. However, the surprisingly high nitrogen and sulfur contents make these microalgae a less favorable feedstock for biofuel production.

The fatty acid profiles show that microalgal oils are similar in fatty acid content to soybean oil, which has been used to produce biodiesel for years. This fact makes microalgal oil a good feedstock for biodiesel production. In obtaining microalgal lipids by extraction, the results showed that the modified Bligh-Dyer extraction method gave a higher extraction yield than the Soxhlet method because it contained the least amount of ash, and all of the brown microalgae strains contained much higher ash contents. Ultimate analysis indicated that both FD and SD mixed marine green microalgae are better options for producing a high heating value biofuel due to their higher carbon content and lower oxygen content. However, the surprisingly high nitrogen and sulfur contents make these microalgae a less favorable feedstock for biofuel production.

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