Extraction and characterization of algal oil from Lake Sebu, South Cotabato: A potential source of biodiesel

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ABSTRACT

Algae is rich in storage lipids and fats that can be converted into biodiesel. This study determined the algal oil from Lake Sebu, South Cotabato as biodiesel source. Samples were prepared at varying treatments and ratio with n-Hexane. The algal oil was extracted and efficiency % were determined. FFA% and Acid Number of the oil extract were identified using chemical titration. FTIR was used for Chemical characterization while GC-MS identified fatty acid and other organics. With the constant volume of solvent and by changing the mass of dried algae, the percent yield of oil increases as the solvent to algae ratio increases. Therefore, biomass ratio with n-Hexane should be 1:3. The IR spectra of the oil extract indicated the presence of functional groups such as amine and carbonyl group of amides, methylene, methyl, and alkene. While, GC-MS showed that the fatty acids found can be a potential biodiesel.

Keywords: Algae, Biodiesel, Extraction Efficiency, Free Fatty Acid, Functional Groups, FAMEs

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1. Introduction

A constant rising worldwide demand of motor and power generation fuels, together with environmental concerns in terms of Green House Gases (GHG), has motivated the scientists and technologists to think about various alternate sources of energy (Singh & Gu, 2010). This explains why despite improvements in the recovery of traditional fossil fuels, more attention needs to be paid to the search for clean and viable alternative renewable energy resources with the prospect of minimizing increases in atmospheric CO₂ by recycling carbon from the atmosphere (Christi, 2017; Huang et al., 2010; Chen et al., 2018).

With the increasing amount of waste originating from human activities comes with the negative impact on the environment and in particular the water quality. Waste streams and lakes, which are rich in carbon, nitrogen, and other minerals, have potential use as a substrate for algae cultivation (Hammouda et al., 2015; Hoffman, 2018).
According to Buijkr (2013) algae are large and highly diverse group of organisms which can be found in almost all ecosystems. Algae are promising feedstock for biodiesel production Scott et al. (2010) and Knothe (2010) and different applications, such as wastewater purification according to (Lundquist et al., 2010; Woertz et al., 2009; Rawat et al., 2011). Furthermore, according to Yun et al. (1997) and Pittman et al. (2011), biogas production, and extraction of value-added compounds for food and pharmaceutical products (Park et al., 1998). An important aspect of biodiesel production is the selection of a suitable algal species (Spolaore, 2016). Selected strains should have two important key characteristics: high biomass productivity as well as adaptation to regional climatic conditions (Mata et al., 2010; Abomohra et al., 2013).

Recent studies of Abou-shanab et al. (2011), Sivakumar et al. (2012) and Yun et al. (2014) proved that algae have inherent advantageous qualities like rapid biomass formation, high lipid content, tolerance for extreme environments, and thus they have generated significantly increased interest as potential feedstock for biodiesel. According to Talebi et al. (2013), a suitable algal candidate for biodiesel production requires not only high lipid productivity, but also suitable fatty acid (FA) composition, as this composition can significantly influence biodiesel fuel properties such as extraction efficiency, kinematic viscosity, specific gravity, cetane number (CN), cloud point, iodine value (IV), long-chain saturated factor (LCFF) and Cold Filter Plugging Point (CFPP), supported by Hoekman et al. (2012).

Lipid production by algae is regulated by various culturing conditions such as carbon source according to Zhao et al. (2012), nitrogen deprivation according to Mujtaba et al. (2012) phosphate limitation as stated by Feng et al. (2012), light intensities according to Sousa et al. (2012) iron content according to Lin et al. (2012), and increasing salinity as stated by Kaewkannetra et al. (2012). Thus, all of these factors influence the growth rate and lipid production of algae.

According to Thomas et al. (2014), algal oils are mostly composed of four unsaturated fatty acids, namely palmitoleic (16:1), oleic (18:1), linoleic (18:2) and linolenic acid (18:3). Saturated fatty acids such as palmitic (16:0) and stearic (18:0) also present with a small proportion. Some special algae could synthesize polyunsaturated fatty acids such as C16:4 and C18:4 in Ankistrodesmus spp., C18:4 and C22:6 in Isochrysis spp., C16:2, C16:3 and C20:5 in Nannochloris spp., C16:2, C16:3 and C20:5 in Nitzschi spp (Clarens et al., 2010).

To further confirm the presence of biodiesel, it requires to be extracted into a particular process. Whereas, Conventional Soxhlet extraction remains as one of the most relevant techniques in the environmental extraction Anderson (2011). Wherein, the sample is placed in a thimble-holder and during operation is gradually flushed with condensed fresh solvent from a distillation Sask. When the liquid reaches an overflow level, a siphon aspirates the whole contents of the thimble holder and unloads it back into the distillation flask, carrying the extracted analytes in the bulk liquid. This operation is repeated until complete extraction is achieved Anderson (2011).

Moreover, to analyze the presence of suitable fatty acids and FAMEs in the algal samples, there are conventional procedures to quantify and enumerate the existing functional groups. For over half a century, GC has played a fundamental role in determining how many components and in what proportion they exist in a mixture. However, the ability to establish the nature and chemical structure of these separated and quantified compounds is ambiguous and reduced, and requires a spectroscopic detection system (Chaintreau, 2011).
Furthermore, higher biomass productivity and lower production costs will also encourage production in the tropics. Therefore, biofuels have the potential to provide opportunities for economic development and improved energy access for developing countries. However, the negative impacts of increased global demand for biofuels are of increasing concern, and include direct and indirect land use change, competition with food production, and land tenure conflicts (Searchinger, 2008).

This study underwent series of procedures to extract and confirm the presence of algal oil as a potential biodiesel. To further analyze the presence of probable functional groups – GCMS ang FTIR were utilized. Samples were taken from the diverse algal bloom in the biggest lake in Lake Sebu, South Cotabato, Philippines and it was processed and extracted at Dole Philippines, Inc., Polomolok, South Cotabato, Philippines.

2. Materials and Methods

2.1 Sample Collection

The algal species from Lake Sebu was collected using the scraping method and caught using a plankton net, and put in a big, clear bottle. To preserve the sample during transportation, it was placed in an ice box filled with ice (Branyikova et al., 2018).

2.2 Sample Extraction

The collected sample were filtered to remove water and the foreign materials were separated from the sample. The sample was dried in an oven for 2 hours under 60°C, and placed in a desiccator for 45 minutes. After which, the dried sample was homogenized using the mortar and pestle (Arun et al., 2017).
2.3 Preparation of Different Treatments

The dried sample was then divided to different mass, namely: 20 g, 20 g, 20 g, 20 g, 15 g, and 10 g, and paired with varying volume of n-Hexane: 20 mL, 30 mL, 40 mL, 30 mL, 30 mL, and 30 mL, respectively. The sample was soaked for 24 to 30 hours and it was squeezed using a mesh bag to obtain the solvent-extract solution. After that, the solution was placed in a 50 mL beaker. Using a hot plate, the solvent was evaporated to get the extract and weighed to get the extraction efficiency (Chen et al., 2015).

2.4 Determining of Percent Yield of Extracted Oil from varying Treatments

After the preparation of varying treatments, soaking of algae, and evaporation of solvent from the varying treatments after 24 hours, the extraction efficiency percentage was obtained by weighing the oil extracted in grams and dividing to the total weight of the dried algal biomass according the specific ratio, then was multiplied to 100 (refer to the formula below). After getting the oil extraction efficiency %, the oil extracted was used for the titration to get the FFA% and AN (Baig et al., 2018).

\[
\text{Oil Extraction Efficiency \%} = \frac{\text{Oil Extracted (g)}}{\text{Dried Algae (g)}} \times 100\%
\]

2.5 Chemical Titration Method for FFA Determination

Extracted oil was transferred in a 250 mL Erlenmeyer flask, added with 50 mL reagent grade methanol, and mixed. Then, 2 mL of 1% phenolphthalein indicator was added, and titrated with standardized 0.1562 M of NaOH and swirled until faint permanent pink appears. The initial and final volume of standardized NaOH used were recorded. The total volume of NaOH used was calculated as follows:

\[
V_{\text{NaOH}} = V_{\text{NaOH}_f} - V_{\text{NaOH}_i}
\]

Free fatty acid % (FFA%) expressed as oleic acid and the acid number (AN) were determined following the equation below. (AOAC Official Method [940.28], 2010).

\[
\%\text{FFA} = \frac{0.1637 \text{ M} \times (V_f - V_i) \times 0.28246}{\text{wt. sample (g)}}
\]

\[
\text{AN} = 1.99 \times \%\text{FFA}
\]

2.6 Soxhlet Extraction Procedure

The dried sample (5 g) was weighed and placed in a thimble for extraction. The thimble was placed in Soxhlet flask and 150 ml of petroleum ether was added. The Soxhlet extraction took up to 4 to 6 hours. After extraction the solvent was removed, by means of a rotary evaporator. The extracted oil was used for the chemical characterization using FTIR and qualitative identification of fatty acid methyl esters and other volatile organics present in the oil using GC-MS (Anderson, 2011; Luque de Castro & da Silva, 1997).
2.7 Chemical Characterization using FTIR

Chemical characterization of the oil extract was conducted using Fourier Transform Infrared Spectroscopy (FTIR) using horizontal attenuated total reflectance accessory. It utilized IRAffinity-1S FTIR Spectrophotometer (Shimadzu) with 12,000 spectra with high stable dynamic alignment mechanism and robust S/N ratio of 30,000:1.

The extract was placed on an attenuated total reflection crystal plate and the IR spectra was then collected. The FTIR spectra of samples were determined in the mid IR range of 400–4000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\). Each spectrum was collected in transmittance mode (Vidyadharani, 2013).

2.8 FAMEs Characterization using GC-MS

Qualitative identification of fatty acid methyl esters (FAMEs) and other volatile organics were determined using Gas Chromatography – Mass Spectroscopic (GC-MS) analysis. The process used the Multi-Dimensional GCMS (Shimadzu) using DB-5 column using helium as the carrier gas (1 mL/min). The temperature at 250 °C and 320 °C with electron ionization (70 eV).

For this analysis, 0.10 ML of the extracted sample was diluted with 2 ML n-Hexane and subjected to GC-MS analysis (Chaintreau, 2011; Flotron, 2013; Kingston & Haswell, 1997).

The sample was injected into a gas chromatograph which volatilizes the sample, then separates the various components of the sample based on size and/or polarity. The separated components then go into a mass selective detector. The resulting mass spectrum allows for the identification of the components using standard reference libraries.

2.9 Statistical Analysis

Kruskal-Wallis H Test was utilized to determine the significant differences between the mean of two sets of varying ratios of volume of n-Hexane to mass of sample, with \(\alpha = 0.05\) which ranks the data to know what ratio was more appropriate to have a more efficient extraction from the algae.

After that, the Mann-Whitney U Test determined the significant difference on the extraction efficiency of sample with two population from algae on the varying ratio of volume of n-Hexane to mass of sample which compared what variation yielded more efficient extraction percentage.

According to McDonald (2014), the said statistical tests were used when there is one nominal variable and one measurement variable in the study. It is considered to be the nonparametric version of the ANOVA Test wherein its measurement variable does not meet the normality assumption of the standard one-way ANOVA.

The aforementioned statistical procedures were conducted electronically, using IBM SPSS\textsuperscript{®} Statistics software to perform nonparametric statistical tests without having to compute for the values manually.

Qualitative approach was then utilized for analyzing the obtained data based on the FFA% and AN of the extracted oil from titration. Moreover, functional groups present in the oil extract determined using FTIR and FAMEs and other volatile organics were also qualitatively identified using GC-MS.
3. Results and Discussion

3.1 Extraction Efficiency of Varying of n-Hexane to Mass of Algae

Table 1 shows the extraction efficiency of varying volume of hexane to mass of algae ratio. Results revealed that a 1:2 ratio in treatment 3A, of constant algae mass to varying volume of n-Hexane, has the highest oil extract of 0.0465g resulting to 0.2322% of extraction efficiency. On the other hand, the treatment 2A, having a ratio of 2:3, showed a 0.0357g oil extract resulting to 0.1785% of extraction efficiency. The 1:1 ratio in treatment 1A showed the lowest extraction efficiency of 0.0417%, having 0.0084g of oil extract. Results showed that increasing the volume of hexane also increases the efficiency % of extraction as well.

<table>
<thead>
<tr>
<th>Table 1. Amount of extracted oil by varying volume of solvent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>1A</td>
</tr>
<tr>
<td>2A</td>
</tr>
<tr>
<td>3A</td>
</tr>
</tbody>
</table>

Note: 1A = 1st treatment with 20g of dried algae soaked in 20mL of n-Hexane; 2A = 2nd treatment with 20g of dried algae soaked in 30mL of n-Hexane; and 3A = 3rd treatment with 20g of dried algae soaked in 40mL of n-hexane

<table>
<thead>
<tr>
<th>Table 2. Amount of extracted oil by varying mass of dried algae.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>4B</td>
</tr>
<tr>
<td>5B</td>
</tr>
<tr>
<td>6B</td>
</tr>
</tbody>
</table>

Note: 4B = 1st treatment with 20g of dried algae soaked in 30mL of n-Hexane; 5B = 2nd treatment with 15g of dried algae soaked in 30mL of n-Hexane; and 6B = 3rd treatment with 10g of dried algae soaked in 30mL of n-hexane

Figure 3. Effect of solvent to algae ratio on the extraction efficiency.
Result showed that a 1:3 ratio in treatment 6B, of constant volume of n-Hexane to varying algae mass, has the highest oil extract of 0.0369g resulting to 0.3687% of extraction efficiency. On the other hand, the treatment 5B, having a ratio of 1:2, showed a 0.0358g oil extract resulting to 0.2386% of extraction efficiency. The 2:3 ratio in treatment 4B showed the lowest extraction efficiency of 0.2317% having 0.0463g of oil extract. It was observed that the percent yield of oil increased as the solvent to algae ratio increased as well.

However, as suggest by Prabakaran et al. (2011) that efficiency of different cell disruption methods in improving lipid extraction varies for different algae species. This ratio is due to green macroalgae that have high content of carbohydrates in the form of cellulose and starch from the lake algae (Hurd et al., 2014).

The effect of solvent to algae ratio on percent yield of extracted oil was shown in Figure 4.0. It was observed that the percent yield of oil increases as the n-Hexane to algae ratio increases, where treatment 1A showed an extraction efficiency of 0.0417%, while treatment 6B showed an extraction efficiency of 0.3687%, which yield 8.84 times higher.

The higher yield of solvent to algae ratio attributed to the excess solvent available to maximize extraction of oil from the algal biomass. Hence, the greater the ratio between solvent to algae biomass, the greater the extraction efficiency (Baig et al., 2018).

### 3.2 Free Fatty Acid

Free fatty acid was determined and expressed as oleic acid by titration with standardized NaOH, which was shown in Table 3. The extraction efficiencies were taken from the chemical titration method in the form of % Free Fatty Acid). Treatment 1A which yielded the highest having 4.78% of FFA. Furthermore, the second highest FFA was treatment 2A, followed by treatment 3A having 2.29 %, treatment 6B of 2.13 %, and treatment 4B of 2.10 %. The free fatty acid determined the acid degree of an oil that could contribute to the possibility of transforming the oil to a low-grade biodiesel synthesis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>VNaOH&lt;sub&gt;i&lt;/sub&gt;</th>
<th>VNaOH&lt;sub&gt;f&lt;/sub&gt;</th>
<th>%FFA</th>
<th>Acid number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>0 mL</td>
<td>2.6 mL</td>
<td>2.60 mL</td>
<td>4.79%</td>
</tr>
<tr>
<td>2A</td>
<td>2.6 mL</td>
<td>4.7 mL</td>
<td>2.10 mL</td>
<td>2.72%</td>
</tr>
<tr>
<td>3A</td>
<td>4.7 mL</td>
<td>7.0 mL</td>
<td>2.30 mL</td>
<td>2.29%</td>
</tr>
<tr>
<td>4B</td>
<td>7.0 mL</td>
<td>9.1 mL</td>
<td>2.10 mL</td>
<td>2.10%</td>
</tr>
<tr>
<td>5B</td>
<td>9.1 mL</td>
<td>11.8 mL</td>
<td>2.70 mL</td>
<td>3.49%</td>
</tr>
<tr>
<td>6B</td>
<td>11.8 mL</td>
<td>13.5 mL</td>
<td>1.70 mL</td>
<td>2.13%</td>
</tr>
</tbody>
</table>

Mean: 2.92% 5.81

Analysis range: 2.10%–4.79% 4.17–9.53

European standard (EN14214): 0.468%–7.14% 0.935–14.27

**Note:** 1A–3A = Constant algae mass and varying volume of n-Hexane; 4B–6B = Constant volume of n-Hexane and varying algae mass

Moreover, the acid number is highly constituted to the %FFA. When the acid number or FFA content of the oil decreases, the diglyceride intermediate and triglyceride content decrease as well. The data above met the specification of EN14214 for biodiesel standard of 0.468–7.14 FFA% and 0.935–
14.27 Acid Number (mg KOH/g oil) (Abubakar et al., 2018 and Yuvarani et al., 2017). In general, the optimum conditions used in the extraction of oil from algae was applicable to produce a biodiesel

### 3.3 Chemical Characterization of Oil Extract using FTIR

Figure 4. FTIR result of the algal oil extract.

Every functional group has its own assigned wave number (cm⁻¹), which was read by the FTIR spectrometer. Occurrence of a peak at specific wavelengths indicated the presence of the assigned functional group, shown in Table 4.

<table>
<thead>
<tr>
<th>Frequency range (cm⁻¹)</th>
<th>Wave number (cm⁻¹)</th>
<th>Assignment</th>
<th>Group and class</th>
</tr>
</thead>
<tbody>
<tr>
<td>3510-3460</td>
<td>3443</td>
<td>NH stretch</td>
<td>Aromatic Primary Amine</td>
</tr>
<tr>
<td>2935-2815</td>
<td>2930; 2897</td>
<td>C-H asym./sym. stretch</td>
<td>Methylene C-H</td>
</tr>
<tr>
<td>1750-1725</td>
<td>1746</td>
<td>C=O stretch</td>
<td>Ester</td>
</tr>
<tr>
<td>1680-1630</td>
<td>1680</td>
<td>C=C stretch</td>
<td>Alkenes</td>
</tr>
<tr>
<td>1470-1430/1380-1370</td>
<td>1470; 1380</td>
<td>C-H asym./sym. bend</td>
<td>Methyl C-H</td>
</tr>
<tr>
<td>1210-1150</td>
<td>1167</td>
<td>CN stretch</td>
<td>Tertiary Amine</td>
</tr>
</tbody>
</table>

Infrared (IR) spectra of the algal oil extract showed an aromatic primary amine (NH stretch) at 3443 cm⁻¹ and tertiary amine (CN stretch) peak of amide at 1167 cm⁻¹, which are considered as nitrogen-based compounds. Moreover, alkene (C=C stretch) peak was also detected in the oil at 1680 cm⁻¹. Long sharp methylene (C-H₂) (C-H asym./sym. stretch) peak was found at 2930 cm⁻¹ and 2897 cm⁻¹, and methyl (C-H asym./sym. bend) peak at 1470 cm⁻¹ and 1380 cm⁻¹ these peaks indicated the presence of hydrocarbons which is one of the main component of biodiesel. In addition, peak found at 1746 cm⁻¹ was assigned to (C=O stretch) of ester which indicated that the extracted oil possibly contains fatty acid methyl esters.

As supported by Vidyadharani (2013), on a range of vibrationally active functional groups including N-H, C=O, -CH₂, -CH₃, C-H, and C=C in algal specimens plays an important role in sequestration of
organic components to synthesize a low-grade biodiesel source. Results revealed that the algal oil contains functional groups similar to the algal oil extract studied by (Meng et al., 2013). Results indicated that the algal oil extract can serve and can be used in biodiesel synthesis.

3.4 GC-MS Analysis

Result of the analysis showed that 18 hydrocarbon-based compounds and fatty acids esters were detected as shown in Figure 5.

![Image of GC-MS result](image.png)

**Figure 5.** GC-MS result of treatment A.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Ret. time</th>
<th>Systematic name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.226</td>
<td>Glucopyranuronamide</td>
</tr>
<tr>
<td>2</td>
<td>6.394</td>
<td>2-Butenedioic acid</td>
</tr>
<tr>
<td>3</td>
<td>10.799</td>
<td>Heptadecane</td>
</tr>
<tr>
<td>4</td>
<td>21.887</td>
<td>Diisooctyl adipate</td>
</tr>
<tr>
<td>5</td>
<td>23.813</td>
<td>1,2-Benzenedicarboxylic acid, diisooctyl ester</td>
</tr>
<tr>
<td>6</td>
<td>30.069</td>
<td>2,4,6-Cycloheptatrien-1-one</td>
</tr>
<tr>
<td>7</td>
<td>33.560</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>8</td>
<td>37.099</td>
<td>Chloest-4-en-3-one</td>
</tr>
<tr>
<td>9</td>
<td>41.796</td>
<td>Bicyclo[4.1.0]hept-4-en-2,3-dicarboxylic acid</td>
</tr>
</tbody>
</table>

The gas chromatogram in Figure 5 revealed a high concentration of 16-Octadecenal, classified as a bio-oil component, indicated by the presence of a long and sharp peak. Alongside with other algal oil characteristics detected by the gas chromatogram, summarized in Table 5, detected peaks of (1) Heptadecane, a volatile oil component; (2) Benzeneethanamine, as a volatile oil; (3) Heneicosane, a hydrocarbon; and (4) 2-Undecanone, 6,10-dimethyl-, an organic compound (colorless oil).

Furthermore, (1) 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, was also detected, classified as an acyclic diterpene alcohol; (2) Tetrapentacontane, 1,54-dibromo-, classified as a hydrocarbon; (3)
2,4-D Butoxyethyl ester, an ester; 1,3,5-Triazin-2(1H)-one, a nitrogen-containing heterocycle; and (4) 4,25-Secoobscurinervan, an organic compound.

Table 6. Functional groups retention time of treatment B (with varying mass of algae and constant 30 mL of n-Hexane) through GC-MS.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Ret. time</th>
<th>Systematic name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.075</td>
<td>16-Octadecenal</td>
</tr>
<tr>
<td>2</td>
<td>5.454</td>
<td>Heptadecane</td>
</tr>
<tr>
<td>3</td>
<td>6.358</td>
<td>Benzeneethanamine</td>
</tr>
<tr>
<td>4</td>
<td>10.812</td>
<td>Heneicosane</td>
</tr>
<tr>
<td>5</td>
<td>13.375</td>
<td>2-Undecanone, 6,10-dimethyl-</td>
</tr>
<tr>
<td>6</td>
<td>17.820</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
</tr>
<tr>
<td>7</td>
<td>21.976</td>
<td>Tetrapentacontane, 1,54-dibromo-</td>
</tr>
<tr>
<td>8</td>
<td>23.682</td>
<td>2,4-D Butoxyethyl ester</td>
</tr>
<tr>
<td>9</td>
<td>27.100</td>
<td>1,3,5-Triazin-2(1H)-one</td>
</tr>
<tr>
<td>10</td>
<td>28.934</td>
<td>4,25-Secoobscurinervan</td>
</tr>
</tbody>
</table>

Note: B = Constant volume of n-hexane and varying algae mass.

Other compounds detected were the following: 2-Butenedioic acid, classified as an omega-dicarboxylic acid; (2) Heptadecane, classified as a volatile oil component; (3) Diisoctyl adipate, classified as a diester organic compound; (4) 1,2-Benzenedicarboxylic acid, diisoctyl ester, an ester; (5) 2,4,6-Cycloheptatrien-1-one, an organic compound; (6) Cholesterol, an organic compound; (7) Choleste-4-en-3-one, a chloestanoid; and (8) Bicyclo[4.1.0]hept-4-en-2,3-dicarboxylic acid, a carboxylic acid as summarized in Table 6.

Results of GCMS analysis of oil extracts, confirmed that the algal oil contains esters, oils, and other volatile compounds which indicated that the oil extracted from algae is a potential for biodiesel synthesis. The extracted oils can be used as a feed stock for liquid fuels, such as biodiesel (Meng et al., 2013).
3.5 Difference of the Extraction Efficiency for Constant Algae Mass with varying volume of n-Hexane.

Since p-value = 0.0385 ≤ 0.05 = α, as shown in Table 7, it rejects the null hypothesis, \( H_01 \). At the α = 0.05 level of significance, there exists enough evidence to conclude that there is no equal yield of extraction efficiency percentage to the constant algae mass and varying volume of n-Hexane ratio.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total N</th>
<th>Test statistics</th>
<th>Degrees of freedom</th>
<th>Asymptotic Sig. ( (2\text{-sided test}) )</th>
<th>decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>2.000</td>
<td>2</td>
<td>0.0385</td>
<td>Reject</td>
</tr>
</tbody>
</table>

Note: A = Constant algae mass and varying volume of n-Hexane

3.6 Difference of the Extraction Efficiency for Constant Volume of n-Hexane with varying Algae Mass.

The same as the analysis of treatment A, the p-value of sample B was 0.0385 ≤ 0.05 = α, as shown in Table 4.7, it rejects the null hypothesis, \( H_02 \). At the α = 0.05 level of significance, illustrated in Table 8, there exists enough evidence to conclude that there is no equal yield of extraction efficiency percentage to the constant volume of n-Hexane and varying algae mass ratio.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total N</th>
<th>Test statistics</th>
<th>Degrees of Freedom</th>
<th>Asymptotic Sig. ( (2\text{-sided test}) )</th>
<th>decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>3</td>
<td>2.000</td>
<td>2</td>
<td>0.0385</td>
<td>Reject</td>
</tr>
</tbody>
</table>

Note: B = Constant volume of n-Hexane and varying algae mass.

4. Conclusion

Statistically confirmed that there was a significant difference in the extraction efficiency of the different treatments. It was observed that increasing the solvent amount also improves the extracted oil efficiency.

Moreover, the percent yield of oil increases as the solvent to algae ratio increases when using the constant volume of solvent and by changing the mass of dried algae. Results showed that the best optimum value for the biomass to n-Hexane ratio was 1:3. Increasing the algae to solvent ratio from treatment 1A to treatment 6B the extracted oil yield was 8.84 times more. It was concluded that the greater the ratio between solvent to algae biomass, the greater the extraction efficiency.

The FFA % and Acid Number of the oil extract met the European specification (EN14214) for biodiesel standard. IR spectra of the algal oil extract showed peaks that indicated the presence of long chain hydrocarbons and esters which were also comparable to the IR spectra of oil extract that as used for biodiesel synthesis. Results indicated that the algal oil extract has a potential and can be used in biodiesel synthesis.

Furthermore, fatty acid methyl esters and other volatile components of the oil extract using GC-MS showed that the oil contains: (1) 16-Octadecenal; (2) Heptadecane; (3) Benzeneethanamine; (4) Heneicosane; (5) 2-Undecanone, 6,10-dimethyl; (6) 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; (7) Tetrapentacontane, 1,54-dibromo; (8) 2,4-D Butoxyethyl ester; (9) 1,3,5-Triazin-2(1H)-one; (10) 4,25-Secoobscurinervan; (11) Glucopyranuronamide; (12) 2-Butenedioic acid; (13) Diisooctyl adipate; (14) 1,2-Benzenedicarboxylic acid, diisooctyl ester; (15) 2,4,6-Cycloheptatrien-1-one; (16)
Cholesterol; (17) Chloest-4-en-3-one; and (18) Bicyclo[4.1.0]hept-4-en-2,3-dicarboxylic acid.

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