Assessing biodiesel quality parameters for wastewater grown *Chlorella* sp.
Samadhan Yuvraj Bagul, Randhir K. Bharti and Dolly Wattal Dhar

**ABSTRACT**

Microalgae are reported as the efficient source of renewable biodiesel which should be able to meet the global demand of transport fuels. Present study is focused on assessment of wastewater grown indigenous microalga *Chlorella* sp. for fuel quality parameters. This was successfully grown in secondary treated waste water diluted with tap water (25% dilution) in glass house. The microalga showed a dry weight of 0.849 g L⁻¹ with lipid content of 27.1% on dry weight basis on 21st day of incubation. After transesterification, the yield of fatty acid methyl ester was 80.64% with major fatty acids as palmitic, linoleic, oleic and linolenic. The physical parameters predicted from empirical equations in the biodiesel showed cetane number as 56.5, iodine value of 75.5 g I₂ /100 g, high heating value 40.1 MJ kg⁻¹, flash point 135 °C, kinematic viscosity 4.05 mm² s⁻¹ with density of 0.86 g cm⁻³ and cold filter plugging point as 0.7 °C. Fourier transform infra-red (FTIR), ¹H, ¹³C NMR spectrum confirmed the chemical nature of biodiesel. The results indicated that the quality of biodiesel was almost as per the criterion of ASTM standards; hence, wastewater grown *Chlorella* sp. can be used as a promising strain for biodiesel production.

**Key words** | biodiesel, fatty acid profile, FTIR, fuel quality, microalgae, wastewater

**NOMENCLATURE**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>FAME</td>
<td>Fatty acid methyl ester</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infra-red</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>ASTM</td>
<td>American Society for Testing and Materials</td>
</tr>
<tr>
<td>CN</td>
<td>Cetane number</td>
</tr>
<tr>
<td>IV</td>
<td>Iodine value, g I₂ /100 g</td>
</tr>
<tr>
<td>CFP</td>
<td>Cold filter plugging point, °C</td>
</tr>
<tr>
<td>HDPE</td>
<td>High density polyethylene</td>
</tr>
<tr>
<td>GCMS</td>
<td>Gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>SV</td>
<td>Saponification value</td>
</tr>
<tr>
<td>DU</td>
<td>Degree of unsaturation</td>
</tr>
<tr>
<td>LCSF</td>
<td>Long chain saturated factor</td>
</tr>
<tr>
<td>HHV</td>
<td>Higher heating value, MJ Kg⁻¹</td>
</tr>
<tr>
<td>FP</td>
<td>Flash point, °C</td>
</tr>
<tr>
<td>KV</td>
<td>Kinematic viscosity, ν, mm²/s</td>
</tr>
<tr>
<td>ρ</td>
<td>Density, g/cm³</td>
</tr>
<tr>
<td>D</td>
<td>Number of double bonds</td>
</tr>
<tr>
<td>MUFA</td>
<td>Mono unsaturated fatty acid</td>
</tr>
<tr>
<td>PUFA</td>
<td>Poly unsaturated fatty acid</td>
</tr>
<tr>
<td>Wt %</td>
<td>Weight percentage</td>
</tr>
<tr>
<td>Mwi</td>
<td>Molecular weight of a fatty acid</td>
</tr>
<tr>
<td>Ni</td>
<td>Percentage of a given fatty acid</td>
</tr>
<tr>
<td>Di</td>
<td>Number of double bonds in a given fatty acid</td>
</tr>
<tr>
<td>Nc</td>
<td>Weighted average number of carbon atoms</td>
</tr>
<tr>
<td>Ndb</td>
<td>Weighted average number of double bonds</td>
</tr>
</tbody>
</table>

**INTRODUCTION**

Microalgal biodiesel has been reported to have an efficient potential to displace petroleum derived fuels without negatively affecting food and crop production as crop-derived biodiesel and bioethanol are deemed unsustainable (Singh & Dhar 2017). Additional advantages of microalgae over land crops or plants as a biodiesel feed stock include cultivation of microalgae in wastewater or seawater, thereby reducing clean water requirements and helping in wastewater treatment. These can utilize nutrients available in waste
waters, thus reducing the pollution load as well (Mata et al. 2010). Usage of waste water can also eliminate competition for fresh water, saves cost of nutrients as these are in abundance in wastewater, provides treatment of wastewater by assimilating organic and inorganic pollutants, and eliminates dance in wastewater, provides treatment of wastewater by for fresh water, saves cost of nutrients as these are in abundance, of the molecules of 100 g of a given oil (Lapuerta for complete saturation, by means of a stoichiometric reaction between triglycerides and alcohol in presence of a catalyst, usually KOH or NaOH (Harun et al. 2006). Biodiesel consists of long-chain alkyl esters, which contain two oxygen atoms per molecule (Graboski & McCormick 1998). The majority of lipid producing microalgae have a lipid profile suitable for biodiesel production in terms of the type and amount produced (Xu et al. 2006); however, the reliability of the biodiesel produced is of concern in utilizing them for any future venture. Characteristics such as ignition quality, cold-flow properties, and oxidative stability are determined by the structure of the FAs. Triglycerides are composed of one mole of glycerol and three moles of FAs, which have different lengths of carbon chain as the number of unsaturated bonds (Sonntag 1979). The important fuel properties of biodiesel that are influenced by the FA profile, and consequently by the structural features of the various fatty esters, are the cetane number (CN), heat of combustion, iodine value (IV), oxidative stability, cold flow properties, the exhaust emissions, viscosity and lubricity (Mittelbach 1996). The iodine number (IN, or IV) is a parameter used to determine the degree of unsaturation (DU) in a vegetable oil or animal fat. This number indicates the mass of iodine (I2) in grams which is required for complete saturation, by means of a stoichiometric reaction, of the molecules of 100 g of a given oil (Lapuerta et al. 2009). Viscosity is known to increase with increasing chain length (Ramirez-Verduzco et al. 2012). The energy content, CN and viscosity of biodiesel are similar to those of petroleum based diesel fuel (Sheehan et al. 1998). Typically, biodiesel from seed oils (rapeseed or soybean) produces 37 MJ/kg, comparable to the energy density of petroleum diesel, with a higher heating value of 42.7 MJ/kg, while biodiesel derived from algae yields 41 MJ/kg (Xu et al. 2006). Flash point (FP) depends, in general, on the source of oil and is not directly linked to the FA composition (Berman et al. 2011). No specifications are available in the USA regarding cold filter plugging point (CFPP), while in the EU it depends on time and location. The longer the carbon chains in the biodiesel, the worse are their low-temperature properties (Wu et al. 2005). Biodiesel fuels are, in general, characterized by higher density than conventional petroleum diesel, which means that volumetrically operating fuel pumps will inject a greater mass of biodiesel than conventional diesel fuel, which will in turn affect the air-fuel ratio, and hence, the local gas temperatures and NOx emissions, as long as the engine retains its diesel-fuel calibration (Mittelbach 1996). Infra Red spectroscopy is used to identify the molecular structures or functional group of the substances. The biodiesel or fatty acid methyl esters (FAMEs) have the ester bond which is linked to a –CH3. Normally, the ester bonds in IR spectral region have peaks in the range of 1,750–1,750 cm⁻¹ and 1,000–1,300 cm⁻¹ for C=O and –CO, respectively. However, it should be noted that acyl glycerols have the same bonds as well. The IR spectral region at around 1,200 cm⁻¹ for –O-CH3 distinguishes between the FAMEs and acylglycerols, and the presence of peak at 1,200 cm⁻¹ indicates the presence of FAMEs (Knothe 2006). In view of this, a supply of secure, equitable, affordable and sustainable energy, preferably from microalgae, has been considered to be vital for future prosperity (Hall et al. 2004).

MATERIALS AND METHODS

Procurement of Chlorella sp. and its maintenance

Microscopically authenticated, unialgal Chlorella sp. was procured from the germplasm collection of Centre for Conservation and Utilisation of Blue Green Algae, Indian Agricultural Research Institute, New Delhi, India and maintained in modified Bold and Basal medium (Bischoff & Bold 1963).

Collection of wastewater

Okhla Sewage Treatment Plant, Delhi, India was selected to collect secondary treated municipal sewage wastewater, and a total of 60 L wastewater was collected with the help of plastic buckets during winter season of 2014–15. The collected waste water was filtered to remove large particles and then stored in three 20-LHDPE containers at 4 °C–6 °C temperature, which helps stop contaminants from breaking down during transit. Physicochemical properties such as ammoniacal nitrogen (NH₃-N), nitrate nitrogen (NO₃-N), total phosphorus and pH were assessed in the collected waste water as per standard procedures (APHA 1998).

Growth of microalga in wastewater

The filtered waste water was diluted with 25% tap water and to this, 10% inoculum of Chlorella sp. from the exponential
growth phase (15th day of incubation) was added. The selected microalga was grown in 15 litre plastic tubs under greenhouse conditions of 25°C temperature and natural sunlight (60.8–67.5 μmol photons/m²/s) for a period of 21 days (Figure 1). After growth in secondary treated sewage water, biomass was harvested and lipid content was determined in dried biomass according to standard protocols (Bligh & Dyer 1959; Sorokin 1975). In order to maintain the batch cultures, these were maintained by inoculating a known volume of fresh medium every 2 weeks for use as seed culture.

**Preparation of FAME**

The lipid sample (up to 10 mg) was dissolved in dry toluene (1 mL) in a test tube. To this, 0.5 M sodium methoxide in anhydrous methanol (2 mL) was added and the solution was maintained at 50°C for 10 min. Glacial acetic acid (0.1 mL) was then added followed by water (5 mL). The esters formed were extracted in hexane (2 × 5 mL), using a Pasteur pipette to separate the layers. The hexane layer was dried over anhydrous sodium sulphate and filtered before the solvent was removed under reduced pressure on a rotary evaporator (Christie 1993) and the prepared sample was subjected to gas chromatography-mass spectrometry (GCMS) analysis.

**Fourier transform infra-red spectrometry**

Extracted lipids from *Chlorella* and FAMEs were dried, mixed with KBr and ground to reduce the particle size to less than 5 mm in diameter. Sample was analysed with Fourier transform infra-red (FTIR) spectrometer (Bruker, Germany) and spectrum was recorded from 4,000 to 650 cm⁻¹.

**Nuclear magnetic resonance spectroscopy**

¹H and ¹³C nuclear magnetic resonance (NMR) of the microalgal lipid and FAME was analysed using a Mass Spectrometer (Bruker 400 ultrashield, Germany) operating at a carbon–¹³ frequency of 67.88 and 100.64 MHz respectively. Spectrum was recorded using 50 mg oil in 0.5 mL chloroform in 5 mm NMR tubes at controlled temperature of 30 ± 0.1°C in the broadband decoupling mode. Full ¹³C spectrum was obtained following acquisition parameters as data points 65 K, spectral with 24,038 Hz, acquisition time 1.36 s, relaxation delay 2 s and 5,000 scan, dwell time 20.80 μs. ¹H NMR: Oil and FAME sample (20 μL) was placed in 5 mm NMR tubes and dissolved in chloroform (0.7 mL) and dimethyl sulphoxide (20 μL), and spectrum was obtained at 400.13 MHz.

**Determination of physical properties of biodiesel**

The quality of biodiesel was assessed in terms of different physical parameters, namely CN, saponification value (SV), IV, DU, long chain saturated factor (LCSF), CFPP, higher heating value (HHV), FP, kinematic viscosity (KV) and density (ρ) of the wastewater grown *Chlorella* sp. with the use of different empirical equations. The CN was estimated by the empirical Equation (1) proposed by Krisnangkura (1986). SV and IV were estimated by the Equations (2) and (3) given by Gopinath et al. (2009).

\[
CN = \frac{46.3 + 5458 \times SV}{SV} - (0.225 \times IV) 
\]

\[
SV = \sum \frac{(560 \times N)}{M} 
\]

\[
IV = \sum \frac{(254 \times D \times N)}{M} 
\]

D is the number of double bonds, M is the molecular mass and N is the percentage of each FA component. The long chain saturated factor was obtained from empirical Equation (4), taking into account the composition of FAs and assigning more weight to the composition of FAs with a long chain. This parameter was correlated with CFPP, using Equation (5) (Ramos et al. 2009).

\[
LCSF = (0.1 \times C16) + (0.5 \times C18) + (1 \times C20) + (1.5 \times C22) + (2 \times C24) 
\]

\[
CFPP = (3.1417 \times LCSF) - 16.477 
\]

where LCSF is the long chain saturated factor; C16, C18, C20, C22 and C24 are the weight percent (wt %) of each of the FAs; and CFPP is the cold filter plugging point.
The DU was calculated from empirical Equation (6), taking into account the amount of monounsaturated and polyunsaturated methyl ester (wt %) present in the microalgae oil (Ramos et al. 2009).

\[
DU = MUFA + (2 \times PUFA)
\]  

\( (6) \)

DU is the unsaturation degree (%), MUFA is the weight percentage of the monounsaturated FAs (wt %) and PUFA is the weight percentage of the polyunsaturated FAs (wt %). The KV \((u, \text{mm}^2/\text{s})\) at 40 \(^\circ\)C and density \((\rho, \text{g/cm}^3)\) of the biodiesel at 20 °C are estimated according to Equations (7) and (8) (Ramirez-Verduzco et al. 2012), and the higher heating value (HHV) is estimated as per Equation (9) (Demirbas 1998).

\[
\ln (u) = \sum Ni (–12.503 \times (2.496 \times \ln Mwi) – 0.178 \times Di)
\]  

\( (7) \)

\[
\rho = \sum Ni \left( 0.8463 \times \left( \frac{4.9}{Mwi} \right) + 0.0118 \times Di \right)
\]  

\( (8) \)

\[
HHV = 49.43 – (0.015 \times IV \times SV)
\]  

\( (9) \)

Here, Mwi is the molecular weight of a FA, Ni is the percentage of the given FA in the biodiesel and Di is the number of double bonds in the given FA (Su & Liu 2011).

Flash point = \((25.362 \times NC + 4.854 \times NDB)\)

\( (10) \)

NC is weighted average number of carbon atoms and NDB is weighted average number of double bonds in the biodiesel. These equations have been utilized to assess the quality of biodiesel produced from microalgae in comparison with biodiesel produced from different vegetable oils (Francisco et al. 2010).

**RESULTS AND DISCUSSION**

**Growth parameters and wastewater characterization**

Waste water collected from Okhla Sewage Treatment Plant, Delhi, India showed a pH of 7.9, ammoniacal nitrogen as 35.0 mg/L, nitrate nitrogen of 3.5 mg/L and total phosphates as 2.5 mg/L, and hence, can be effectively used for the growth of microalgae as wastewater including municipal (urban) sewage wastewater and agricultural manure wastewater have been reported to be potential sustainable growth media for microalgae (Pittman et al. 2011). Secondary treated waste water after diluting with tap water (75:25) was used for the growth of *Chlorella* sp., which showed an average dry weight of 0.849 g/L with a lipid content of 27.1% on dry weight basis, while a few studies have reported a lipid content of 25% in waste-grown algae cultures (Enssani 1987). Municipal sewage water has been reported to support the mixotrophic and heterotrophic growth of microalgae, as it contains organic compounds such as volatile acids, non-volatile soluble acids, FAs, amino acids and carbohydrates (Zhang et al. 2008). These organisms have a high capacity for inorganic nutrient uptake and can efficiently remove significant amounts of nitrogen and phosphorus required for synthesis of proteins, nucleic acids and phospholipids (Rawat et al. 2011). *Euglena* sp. originally isolated from the sewage treatment plants showed good lipid content of 24.6% (w/w) and efficient nutrient uptake within a short span of eight days with profuse biomass productivity (132 mg/L/d) (Mahapatra et al. 2013). Other microalgal genera, namely *S. quadricauda, Nannochloropsis* sp., *Chlorella* sp. and *Oscillatoria* sp., contained a lipid content of 18.4%, 29.2%, 18.7% and 5.0% respectively under photoautotrophic cultivation without any stress (Repka et al. 1998). A total of 17 microalgal strains isolated from water bodies including wastewater from Minnesota grew well in centrate (highly concentrated municipal) wastewater. Out of these, five strains, namely *Chlorella* sp., *Heynigia* sp., *Hindakia* sp., *Micractinium* sp., and *Scenedesmus* sp., were promising regarding their ability to adapt to centrate municipal wastewater and showed high growth rates (0.455–0.498/d) with high lipid productivities (74.5–77.8 mg/L/d) (Zhou et al. 2012).

**FA composition**

Total yield of FAMEs in wastewater grown *Chlorella* sp. was 80.64%, which consisted of ten FAs. Major FAMEs formed included methyl hexadecanoic acid (palmitic acid, 36.97%), methyl octadecatrienoic acid (linolenic acid, 12.45%), and methyl 9,12-octadecadienoic acid (linoleic acid, 9.88%) (Table 1). FAME analysis of *A. protothecoides* UMN280 lipids showed predominance of C16/C18 FAs, accounting for over 94% of total FA, making it suitable for high-quality biodiesel production (Zhou et al. 2012). The feasibility of cultivating *Chlorella vulgaris* was evaluated in wastewater having high concentration of ammoniacal nitrogen. Increase in concentration of NH\(_4\)-N from 17 to 207 mg L\(^{-1}\) yielded additional short-chain and saturated FAs (He
Varying cultural conditions also influenced FA composition, with linolenic acid (C18:2) predominating. FAME profile of *Desmodesmus* sp. cultured in wastewater showed that the palmitic acid (C16:0) was the most abundant (Samori et al. 2013). Over 80% of total FA profile is considered as an ideal source of lipid for biodiesel production (Liu et al. 2011). Converti et al. (2009) reported that microalgal oil contains high values of palmitic acid and the concentration of linoleic acid met the requirements of the European legislation for biodiesel. The present study clearly indicated the suitability of biodiesel from wastewater grown *Chlorella* sp. as per the FAME profile data, and it can be considered as a promising microalga as a source of lipids for biodiesel production.

**Prediction of biodiesel quality parameters based upon FAME profile**

The CN is the dimensionless number of the ignition quality of a diesel fuel and is a prime indicator of biodiesel quality. In the present study, CN was found to be 56.5, and standard values for CN indices of ASTM D6751 (ASTM 6751 2002) for biodiesel fuel require a minimum CN of 47, while in the European standard (EN 14214) (UNE-EN 14214 2003) and the Australian standard (AMEH 2003), a value of 51 is reported. The CN values are reported to strongly affect engine performance parameters such as combustion and exhaust emissions, and higher CN has been correlated with lower nitrogen oxides exhaust emissions (Ladommatos et al. 1996). Branching and chain length affect CN, as the number becomes smaller with decreasing chain length and increase in branching. Long, unbranched chains of FAs produce a good conventional biodiesel fuel which represents the ignitability of fuel, particularly critical during cold starting conditions (Clothier et al. 1993). Low CNs lead to long ignition delay, which is a delay in fuel injection and start of combustion. Consequently, the lower the CN value, the more abrupt is the premixed combustion phase, which leads to higher combustion noise radiation (Giakoumis et al. 2012). Higher CNs promote faster auto ignition of the fuel, and often lead to lower NOx emissions (Peterson et al. 2000). High fuel IV indicates the possibility of polymerization resulting in deposit formation (Graboski & McCormick 1998). The present study showed an IV of 75.5 g I2/100 g, and biodiesel used in compression ignition engines should have a maximum value of IV of 120 as per European norms. High heating value of the wastewater grown *Chlorella* sp. was 40.1 MJ/kg, which met the ASTM standard value of more than 35 MJ/kg, and no specifications have been reported for the biodiesel heating value in the EU. When the higher heating value increases, fuel consumption decreases (Knothe et al. 2008). Higher heating value increases with an increase in chain length and decreases with an increase in unsaturation. The KV estimated in this study was found to be 4.05 mm2/s, while in ASTM standard; the value is given as 1.9–6.0 mm2/s, and the density of 860 kg/m3 in *Chlorella* was within the range of ASTM and EU standards. The FP is a measure of the temperature to which a fuel must be heated such that the mixture of vapor and air above the fuel can be ignited, and FP varies inversely with the fuel’s volatility. FP of 135 °C estimated in the present study met the standards of the EU (120 minimum) and ASTM (93 minimum).

The low temperature flow of the biodiesel is another important quality parameter when saturated molecules are present and crystallization may occur at temperatures within the normal engine operation range. Therefore, when temperatures are low enough, these crystals grow rapidly and agglomerate, clogging fuel lines and filters (Ramos et al. 2009; Sarin et al. 2009). The study showed a CFPP value of 0.7 °C (Table 2). He et al. (2013) reported that when a liquid biodiesel is cooled, the stearic and palmitic acid methyl esters are the first to precipitate, and therefore typically constitute a major share of material recovered from clogged biodiesel fuel filters. *S. obliquus* biodiesel, which is rich in stearic acid (19.54%), showed the highest CFPP (He et al. 2013). Different physical parameters of wastewater grown *Chlorella* sp. were compared with ASTM 6751 and EN 14214 standards of biodiesel, and from this study, physical properties met all the criteria of

**Table 1 | FA composition of wastewater grown Chlorella sp. by GCMS**

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Retention time</th>
<th>Methyl esters</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.758</td>
<td>Myristic acid</td>
<td>1.09</td>
</tr>
<tr>
<td>2</td>
<td>18.540</td>
<td>Pentadecanoic acid</td>
<td>2.89</td>
</tr>
<tr>
<td>3</td>
<td>18.882</td>
<td>Stearic acid</td>
<td>3.53</td>
</tr>
<tr>
<td>4</td>
<td>19.133</td>
<td>Oleic acid</td>
<td>8.4</td>
</tr>
<tr>
<td>5</td>
<td>21.004</td>
<td>Palmitic acid</td>
<td>36.97</td>
</tr>
<tr>
<td>6</td>
<td>22.166</td>
<td>Palmitoleic acid</td>
<td>3.77</td>
</tr>
<tr>
<td>7</td>
<td>22.652</td>
<td>Margaric acid</td>
<td>0.86</td>
</tr>
<tr>
<td>8</td>
<td>26.600</td>
<td>Linoleic acid</td>
<td>9.88</td>
</tr>
<tr>
<td>9</td>
<td>28.224</td>
<td>Linolenic acid</td>
<td>12.45</td>
</tr>
<tr>
<td>10</td>
<td>31.187</td>
<td>Arachidonic acid</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>80.64</td>
</tr>
</tbody>
</table>
the US and EN biodiesel standards. Therefore, wastewater grown *Chlorella* sp. could be a suitable option for biodiesel production and can contribute to lowering the cost involved in the complete process.

**FTIR and NMR**

In fats and oil, most of the peaks and shoulders of the spectrum are attributable to the functional groups (Silverstein & Webster 1998). FTIR spectrum generated for wastewater grown microalga showed a characteristic band of biodiesel at 1,743 cm\(^{-1}\), which is assigned to C = O ester; 801 and 805 cm\(^{-1}\) are assigned to C-C group, 1,459 cm\(^{-1}\) to C-C

![Figure 2](https://iwaponline.com/wst/article-pdf/76/3/719/451450/wst076030719.pdf)

**Figure 2** | FTIR spectrum of biodiesel from wastewater grown *Chlorella* sp.

![Figure 3](https://iwaponline.com/wst/article-pdf/76/3/719/451450/wst076030719.pdf)

**Figure 3** | \(^1\)H NMR spectrum of biodiesel from wastewater grown *Chlorella* sp.
group, and 2,854, 2,924 and 2,963 cm$^{-1}$ assigned to C-H group (Figure 2). Maity et al. (2014) studied FTIR of Leptolyngbya lipid, and they assigned the infra red bands based on the correlation with FAs, esters and triglycerides. 1H NMR spectroscopy study reveals the presence of monoglyceride as per the peak of 3.6 ppm, and this is reported by earlier studies as well (Kumar et al. 2011). The unsaturated hydrogen appeared in the region of 5.39 ppm and can be used to calculate the IV of the algal extract (Sarpal et al. 1994), and the other signals at 2.27–2.34 ppm are due to $-\text{CH}_2-$ attached to carbonyl group (Figure 3). 13C NMR resonates ester signal in triglyceride at 169.1 ppm, and signal at 129.7 ppm indicates presence of hydroxyl FAs (Figure 4).

**CONCLUSIONS**

The present study indicated feasibility of wastewater grown *Chlorella* sp. isolated from aquatic water body of India in secondary treated waste water. Harvested biomass on the 21st day of incubation showed a lipid content of 27.1%. FAME profile, as well as physical parameters predicted from empirical equations, was in accordance with the ASTM standards for biodiesel quality. 1H NMR and 13C NMR confirmed the structure of the biodiesel compound. FTIR study identified the functional group present in the sample. From the present study, it can be concluded that secondary treated waste water with dilution can be a potential source for biomass production of microalgae, which can be exploited for biodiesel production. Additional benefit using waste water as cultivation medium can minimize the cost of nutrients required for microalgae cultivation.

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