Fuel Processing Technology 120 (2014) 79–88

Contents lists available at ScienceDirect

Fuel Processing Technology

journal homepage: www.elsevier.com/locate/fuproc

Production and characterization of biodiesel from algae

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ARTICLE INFO

Article history:
Received 7 August 2013
Received in revised form 5 December 2013
Accepted 5 December 2013
Available online 4 January 2014

Keywords:
Microalgae
Spirulina
Chlorella
Pond water algae
Transesterification
Biodiesel

ABSTRACT

The feasibility of biodiesel production from microalgae as third generation biodiesel feedstock was studied in the present investigation. The studies were conducted to evaluate the growth patterns of the algae species i.e. *Spirulina*, *Chlorella* and pond water algae. The oil was extracted from the algae biomass and then transesterified. Simultaneous extraction and transesterification were also studied using different solvents. Maximum biodiesel yield was obtained using simultaneous extraction and transesterification using hexane as a solvent. The systematic characterization of algae biomass, algae oil and algae biodiesel was carried out to establish the potential of microalgae for biodiesel production.

1. Introduction

The demand for alternative fuels has increased in the past several years. Several substitutes have come into existence in the recent years and many more are on their way to get established as a sustainable fuel alternative [1]. Biodiesel is gaining importance as one of the most important substitutes for the depleting fossil fuels. There are many advantages of using biodiesel as an alternative form of energy [2,3]. It can be used as such in the diesel engine without any engine modification indicating that it has comparable physical and chemical properties as conventional diesel. The combustion properties of biodiesel are also very close to those of petroleum diesel [4]. Biodiesel is highly biodegradable [5] and is nontoxic as well as renewable. The exhaust of biodiesel during combustion has lesser carbon monoxide, hydrocarbon, particulate matter and sulfur dioxide as compared to those of petro-diesel [6–9]. However, the problems like increased NO_x emissions [10,11], poor cold flow and poor oxidative stability [12] need to be solved. Therefore, a lot of research is focused on control of these NO_x emissions [13–16].

Biodiesel can be derived from edible oil seed crops such as sunflower, palm, rapeseed, soybean, coconut, etc. which are considered as first generation biodiesel feedstocks. However, use of such feedstocks for biodiesel production has faced problems as they disturb the overall worldwide balance of food reserves and safety. The non-edible seed crops of jatropha, karanja, jojoba, mahua and waste cooking oil, grease, animal fats, etc. have gained importance in the last few years as second generation feedstocks for biodiesel production. However, these second generation feedstocks are not sufficient to entirely substitute the present transportation needs.

Recent focus is on microalgae as the third generation feedstock. Using microalgae has several advantages like high photosynthetic efficiency and higher biomass production [17]. Microalgae do not compete for land and can grow anywhere, even in brackish saline water. The current research efforts have been concentrated on increasing lipid content in microalgae [18–20] and culturing of algae [21–24]. In order to establish the potential of microalgae biomass as an alternative for biodiesel production, more concentrated attempts are needed for detailed characterization of algae biomass, algae oil and algae biodiesel as very little information in literature is available on the same.

The present study dealt with three different species of algae i.e. *Chlorella*, *Spirulina* and pond water algae in order to assess their potential for biodiesel production. The natural pond water algae biomass is expected to be a cheaper feedstock for biodiesel production as compared to pure cultures of *Chlorella* and *Spirulina*. The growth patterns of the three algae species were studied with an aim to determine the maximum productivity of algae species. The algae biodiesel production was attempted via oil extraction and transesterification both in single stage and two stage reactor units in order to get the maximum biodiesel yield. The present work investigated the usefulness of techniques like FTIR, NMR, GC and proximate and elemental analyses to understand the chemical properties of algae biomass, algae oil and algae biodiesel. The fuel properties of algae biodiesel were also investigated. The results were then compared with that of karanja biodiesel and conventional diesel in order to establish the potential of algae biomass for biodiesel production.

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http://dx.doi.org/10.1016/j.fuproc.2013.12.003

2. Materials and methods

2.1. Microalgae, growth medium and conditions

Spirulina platensis and *Chlorella pyrenoidosa* used in the present study were procured from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune-Maharashtra, India. The algae sample from a local pond was also collected from India gate, New Delhi, India. All the three samples were maintained in conical flasks containing 50 mL sterile BG11 media [25]. Proper shaking conditions were also maintained at 120 rpm and 24 °C for half an hour and then placed in the direct sunlight. Regular sub culturing was performed after every 15–18 days. In a growth chamber, the cultures were incubated under illumination (2000 lx) at 25 ± 1 °C with light: dark cycles of 12:12 h for 15 to 18 days with bubbling air at normal pressure required to maintain stirring of the cultures. With the concern for large scale cultivation of algae, growth was also attempted in tap water without BG 11 medium.

2.2. Growth evaluation

Growth of all the three species was monitored both in the presence and absence of BG 11 media for the total period of 27 days. Growth of the species was evaluated based on two parameters: (i) chlorophyll a content and (ii) dry cell weight.

(i) *Chlorophyll a content*: Chlorophyll a was extracted and estimated using the procedure used by Chinnaswamy et al. [26]. For this, a known volume of algae biomass was suspended in the medium and centrifuged at 8000 rpm for 10 min. Biomass collected after centrifugation was again suspended in a known volume of methanol. This methanol and algae biomass suspension was then immersed in the water bath for half an hour at 60 °C in order to extract the chlorophyll from the biomass. After the stipulated time, the chlorophyll concentration in the above suspension was spectrophotometrically determined using UV visible spectrophotometer (Systronics117). The absorbance values were then substituted in Eq. (1) used for chlorophyll estimation [27]:

$$\text{Chl a}\left(\frac{\mu g}{ml}\right) = 16.29\left(A^{665.2} - A^{750}\right) - 8.54\left(A^{652} - A^{750}\right) \quad (1)$$

where A^{750}, $A^{665.2}$, A^{652} are referred as the absorbance of algae biomass-chlorophyll suspension in methanol at 750, 665.2 and 652 nm, respectively.

(ii) *Dry cell weight*: In order to evaluate the growth with respect to dry cell weight, a known volume of algae biomass growing in the suspension was taken and centrifuged at 8000 rpm for 10 min. The collected biomass was washed with distilled water to remove the salts present. This biomass was then filtered on the pre-weighed filter paper and dried overnight at 60 °C and then weighed.

2.3. Oil extraction and biodiesel production

The acid values of *Spirulina* algae oil and pond water algae oil obtained in the present study were 9.42 mg KOH/g and 8.86 mg KOH/g respectively. The acid values were quite high and thus acid catalyzed transesterification was done in the present investigation. Acid based transesterification of algae oils also has been reported effective for biodiesel production [28].

(i) *Oil extraction followed by transesterification* (*two stage process*): The dried algae biomass after pulverization was subjected to Soxhlet extraction using hexane as a solvent at 56 °C. The solvent phase was separated from oil phase by distillation.

The lipids separated from the solvent were then subjected to transesterification process for biodiesel production. For transesterification, lipids were treated with methanol along with conc. H_2SO_4 at 60 °C for 1 h. Proper mixing was maintained in the reactor during the process. After the completion of the reaction, products were allowed to cool at room temperature. Water was then added to the reactor and the mixture was transferred to a separating funnel. Phase separation was observed. Lower phase of biodiesel was collected and washed with distilled water. The percentage yield of biodiesel was calculated using the Eq. (2):

$$\text{Yield of biodiesel } (\%) = \frac{\text{Grams of biodiesel produced}}{\text{Grams of the oil used}} * 100 \quad (2)$$

(ii) *Simultaneous oil extraction and transesterification* (*single stage process*): The dried algae biomass was added to the reactor after pulverization. The solvent and methanol along with the catalyst (conc. H_2SO_4) were also added to the reactor. The temperature was maintained at 60 °C for 1 h with mixing. After the reaction was completed, the products were allowed to cool down to room temperature and distilled water was added to the mixture. The products were transferred to the separating funnel which immediately resulted in the formation of two layers. The upper solvent layer with biodiesel was separated and subjected to distillation for biodiesel recovery. Percentage yield of biodiesel was then determined using the above Eq. (2). The effect of different solvents (chloroform, hexane, and no solvent) on biodiesel yield was also studied using the similar procedure.

2.4. Characterization

2.4.1. Proximate analysis

The moisture, volatile matter and ash content of the dried algal biomass of the three species were determined according to ASTM protocol.

2.4.2. CHNS analysis

The elemental analysis of carbon, hydrogen, nitrogen and sulfur of dried algae biomass, algae oil and biodiesel was carried out using CHNS analyzer (Elementar Vario El Cube) and the oxygen content was calculated by difference.

2.4.3. Proton nuclear magnetic resonance (^1H NMR)

The ^1H NMR analysis of algae oil, algae biodiesel, karanja biodiesel and diesel was conducted using Bruken Spectrospin 300 operating at 300 MHz. The samples were prepared in $CDCl_3$ with the ratio of 1:1 by volume in 5 mm NMR tube and TMS (tetramethylsilane) was used as an internal standard.

2.4.4. ^{13}C nuclear magnetic resonance (^{13}C NMR)

The ^{13}C NMR analysis of algae oil, algae biodiesel, karanja biodiesel and diesel was performed on a Bruken Spectrospin 300 operating at 300 MHz. The samples were prepared in $CDCl_3$ with the ratio of 1:1 by volume in 5 mm NMR tube and TMS (tetramethylsilane) was used as an internal standard.

2.4.5. Fourier transform-infrared (FTIR) spectroscopy

The FTIR spectra of dried algal biomass, algae oil and biodiesel were obtained using FTIR spectrometer (Nicolet 6700) at room temperature. The dried algal powder was mixed with potassium

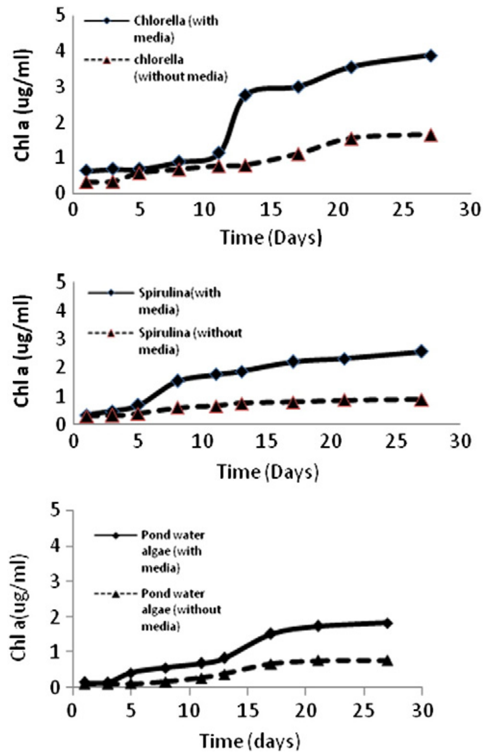

Fig. 1. Chlorophyll a content with time for *Chlorella*, *Spirulina* and pond water algae.

bromide (KBr) powder and pressed into pellets before analysis. The liquid samples of algae oil and biodiesel were sandwiched between the two KBr pellets and then introduced in the spectrometer.

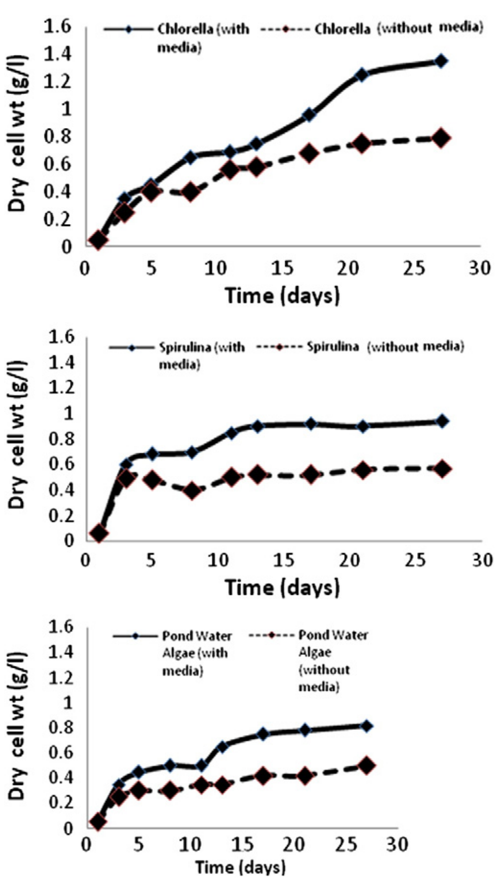

Fig. 2. Dry cell weight with time for *Chlorella*, *Spirulina* and pond water algae.

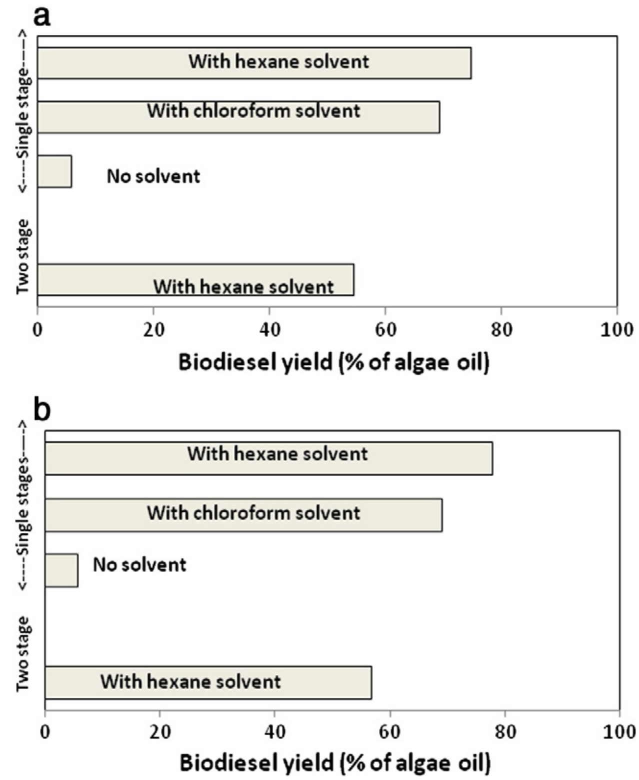

Fig. 3. Effect of solvents on biodiesel yield of (a) pond water algae and (b) *Spirulina* using two stage process and single stage process.

2.4.6. Gas chromatography (GC) analysis

The composition of algae biodiesel (fatty acid methyl esters) produced was determined by using well established GC analysis. Supelco™ 37 Component fame mix was used as a standard for identification and quantification of the peaks obtained in the biodiesel sample on Shimadzu QP-2010 Plus with SP-2560 column (100 m × 0.25 mm × 0.20 μm) and flame ionization detector. A sample of 0.6 μl (0.5 mg of algae biodiesel in 1 ml of hexane) was injected under the split mode of 80:1 and injector temperature was maintained at 260 °C using nitrogen as a carrier gas. The oven was kept at 140 °C for 5 min and then heated up to 240 °C at the rate of 4 °C/min with the holding time of 20 min. The detector temperature was set to 270 °C. The fatty acid methyl ester peaks in the biodiesel sample were identified on comparison with the peaks obtained in the GC chromatogram for the above standard used.

2.4.7. Fuel properties

The characteristic fuel properties of *Spirulina* biodiesel and pond water algae biodiesel were evaluated using standard methods and results were then compared to ASTM D 6571 and EN 1424 standards. The following fuel properties were evaluated: density (specific gravity bottle), viscosity (Fungilab rotational viscometer), acid number (titrimetric analysis), calorific value (RSB Rajdhani digital bomb calorimeter), pour point (assembled pour point tester), flash point (Pensky-martens flash point tester-Biomate) and copper strip corrosion value (assembled copper corrosion set up).

Table 1
Proximate analysis (wt.%).

Algae species	Moisture	(% air-dried) volatile matter	Fixed carbon	Ash	(% dry-ash-free) volatile matter	fixed carbon
Spirulina	5.20	74.41	7.73	12.66	90.59	9.41
Chlorella	11.11	70.14	8.85	10.20	89.13	10.87
Pond water algae	4.01	64.60	6.40	25.00	90.99	9.01

Table 2
CHNS analysis (daf) (wt.%).

Algae species	C	H	N	S	O (by difference)
Pond water algae biomass	46.09	6.22	9.70	0.64	37.35
Spirulina biomass	48.10	6.97	10.14	0.66	34.13
Chlorella biomass	51.33	7.90	9.80	0.59	30.38
Pond water algae oil	59.94	11.57	0.11	0.31	28.37
Spirulina oil	66.73	12.40	0.50	0.16	20.21
Pond water algae biodiesel	71.49	11.00	0.31	0.19	17.01
Spirulina biodiesel	78.44	12.04	0.20	0.08	9.23
Karanja biodiesel	78.10	11.39	0.07	0.06	10.39

3. Results and discussion

3.1. Growth evaluation

Figs. 1 and 2 show the growth pattern of all the three species studied for 27 days in the presence and absence of growth media (tap water). In Fig. 1, the gradual increase in chlorophyll a content with time indicates the growth of algae in all the three cases. However, while comparing the growth in BG 11 medium and in tap water alone (without the media), the chl a content of all the algae species was found to be lower in tap water than that obtained in BG 11 medium. The figure also indicates the slower growth rate of all the species in tap water. On comparison, *Chlorella* was observed to have the highest chl a content (3.88 μg/ml) followed by *Spirulina* (2.56 μg/ml) and pond water algae (1.84 μg/ml) after 27 days in BG media.

Growth of the species was also monitored for a total period of 27 days by measuring the dry cell weight content. Gradual increase in dry cell content (Fig. 2) with time indicates the growth of algae in all the three cases in both BG 11 media and without the media; however, growth was found to be slower in the absence of the media. Comparing the three species, *Chlorella* was observed to have the highest dry cell weight content (1.35 g/l with media). On the other hand, *Spirulina* and pond water algae had nearly the same dry cell weight content of 0.94 g/l and 0.82 g/l with media respectively at the end of 27 days.

The figures show that there was gradual increase in the growth of algae species up to 15–18 days after which growth was almost stable. Pond water algae have the potential to grow almost at the same rate as that of *Spirulina*. Also, pond water algae grow naturally in open ponds in the presence of natural nutrients available to them. The utilization of such pond water algae is expected to reduce the overall process cost and hence assumes great importance for biodiesel production. Further studies were, therefore, conducted with pond water algae and results were compared with those of *Spirulina*, rather than using *Chlorella*, inspite of its having the maximum chlorophyll content and dry cell weight and higher growth rate. *Chlorella* is expensive and is a preferred feedstock for food supplement.

3.2. Biodiesel production

The biodiesel yield obtained via a two stage process of oil extraction followed by transesterification was compared with that obtained by simultaneous oil extraction and transesterification, a single stage process

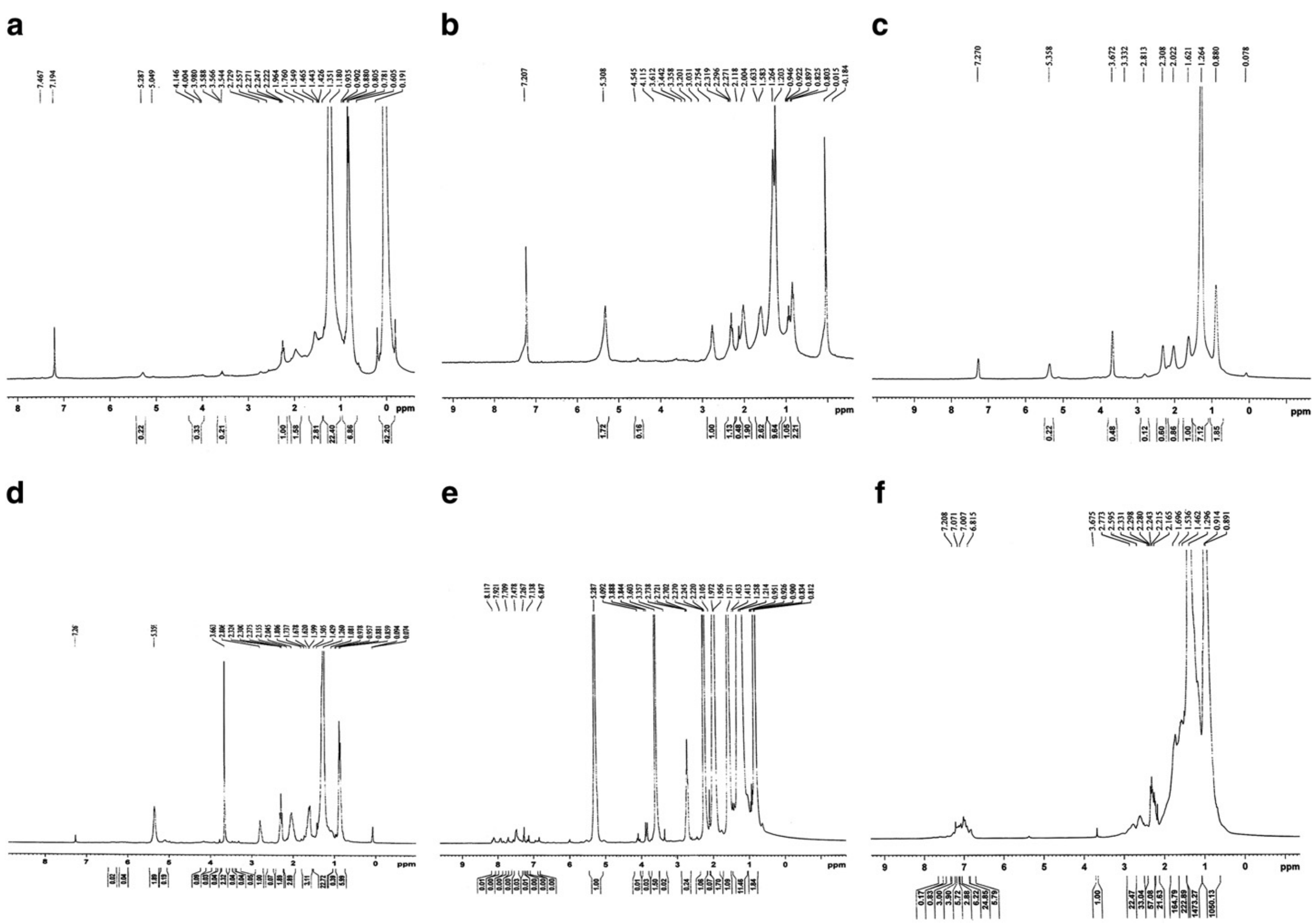

Fig. 4. [1]H NMR spectra of (a) pond water algae oil, (b) *Spirulina* algae oil, (c) pond water algae biodiesel, (d) *Spirulina* algae biodiesel, (e) Karanja biodiesel, (f) diesel.

as shown in Fig. 3 for *Spirulina* and pond water algae. The biodiesel yield was expressed in the terms of relative weight of biodiesel obtained to that of oil present in algae biomass. It was observed that the single stage process resulted in higher biodiesel yield compared to two stage process. Comparing the effect of different solvents on the biodiesel yield, hexane was observed to give higher yield than chloroform. Hexane being more non-polar as compared to chloroform, lipids get more effectively dissolved in hexane than in chloroform ('Like dissolves like' rule). Drastic reduction in biodiesel yield was observed when no solvent was used indicating that solvent is necessary for biodiesel production. Similar trends were obtained both for *Spirulina* and pond water algae. The maximum yield of pond water algae biodiesel and *Spirulina* algae biodiesel obtained using hexane in a single stage process was 74.60% and 79.50% respectively.

3.3. Characterization

3.3.1. Proximate analysis

The proximate analysis gives the information about percentage of moisture, ash, fixed carbon and volatile matter contents of the algae biomass. Table 1 shows the proximate analysis of air-dried algae biomass for the three algae species. Comparing the results obtained from the proximate analysis, the ash content in pond water algae was found to be 25% as compared to 12.66% of *Spirulina* and 10.20% of *Chlorella*. The higher ash content in pond water algae compared to pure cultures of *Chlorella* and *Spirulina* is due to the incorporation of natural inorganic substances into the biomass matrix during its growth. Pond water algae were observed to have comparable volatile matter i.e. 91% as compared to 90.59% of *Spirulina* and 89.13% of *Chlorella* on dry-ash-free basis. This indicates similar potential in all three species on dry-ash-free basis. Moisture content (4.01%) in pond water algae (air-dried) was found to be lower as compared to 11.11% in *Chlorella* and 5.20% in *Spirulina*. Low moisture content helps in better oil extraction resulting in higher yields. Proximate analysis shows more comparable results of *Spirulina* and pond water algae; therefore, further studies were continued with these two species.

3.3.2. CHNS analysis

The CHNS analysis (Table 2) shows that the elemental composition of pond water algae biomass and its oil has close resemblance with that of *Spirulina*. On comparing the elemental percentage of algae oil and its biodiesel, an increase in the percentage of carbon and hydrogen was observed in algae biodiesel in both the cases, which depicts the higher heating value of algae biodiesel. The decrease in the percentage of sulfur, nitrogen and oxygen was also observed in biodiesel. The lower the nitrogen and sulfur percentages in the biodiesel, the lesser will be the exhaust emissions (NO_x and SO_2) while using it in a diesel engine. The lower the oxygen percentage, the lesser will be the oxidative degradation of biodiesel, indicating higher stability of biodiesel. Further the elemental composition of algae biodiesel was compared and was found to have close resemblance with karanja biodiesel which has been established as a potential substitute for diesel [16].

3.3.3. ^1H NMR

NMR is one of the most useful techniques to explain the structure of chemical compounds. Fig. 4a and b shows NMR spectra of pond water algae oil and *Spirulina* algae oil respectively. On comparison of the NMR spectra (Fig. 4c and d) of their respective biodiesel, a characteristic peak at 3.6 ppm could be observed in the biodiesel spectra due to methyl group of ester. This peak was absent in the NMR of algae oil. The NMR spectra of algae biodiesel was compared with that of karanja biodiesel (Fig. 4e) which also showed the peak at 3.6 ppm. Other peaks observed

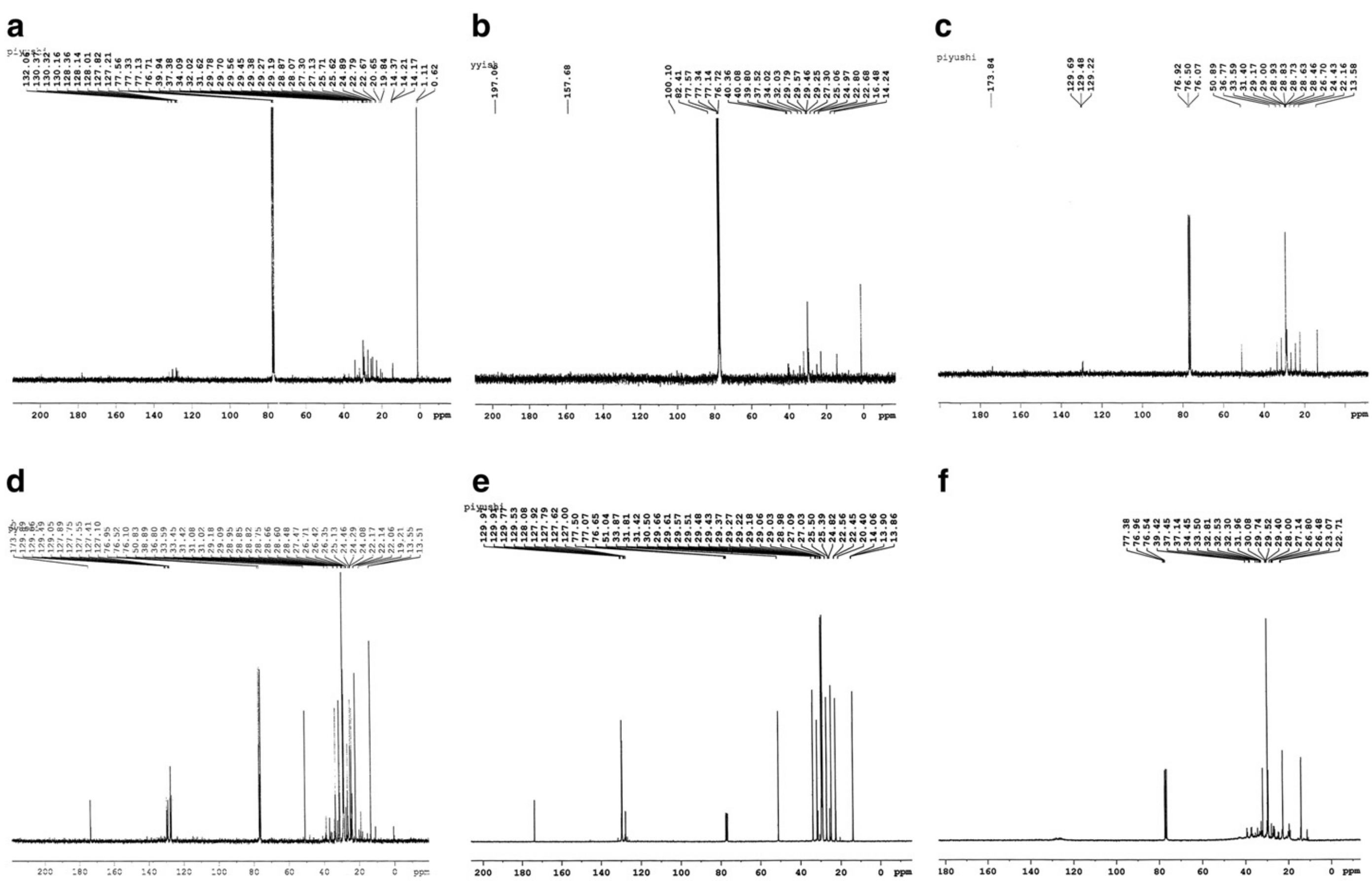

Fig. 5. ^{13}C NMR spectra of (a) pond water algae oil, (b) *Spirulina* algae oil, (c) pond water algae biodiesel, (d) *Spirulina* algae biodiesel, (e) Karanja biodiesel, (f) diesel.

in the [1]H NMR spectra of algae biodiesel and karanja biodiesel were at 0.8 ppm (triplet, due to terminal methyl hydrogens), a strong signal at 1.2 ppm (strong singlet, due to the backbone methylenes of carbon chain), a multiplet at 1.6 ppm (multiplet, due to beta methylenes proton), 2.3 ppm (triplet, due to alpha methylene proton to ester), and some peaks because of unsaturation at 2.0 ppm (alpha methylene group to one double bond), 2.8 ppm (alpha methylene group to two double bonds) and 5.3 ppm (olefinic protons). The presence of peaks at 3.6 ppm and 2.3 ppm confirmed the presence of methyl esters in the sample. [1]H NMR of diesel (Fig. 4f) showed the group of peaks at 0.8–3 ppm from aliphatic hydrogens and another cluster at 6.8–7.2 ppm range from aromatic hydrogen. [1]H NMR spectra of *Spirulina* and pond water algae biodiesel were similar to that of karanja biodiesel supporting the fact that algae biodiesel has the potential as an alternative fuel.

3.3.4. ^{13}C NMR

The ^{13}C NMR spectra of pond water algae oil (Fig. 5a) and *Spirulina* oil (Fig. 5b) were compared with ^{13}C NMR of their respective biodiesel (Fig. 5c and d). ^{13}C NMR spectra of algae biodiesel were observed to have characteristic peaks near 174 ppm and 50.83 ppm related to carbon of carbonyl ester and methoxy group respectively. These peaks give an indication of conversion of algae oil to biodiesel. Several peaks in the range of 127.8–129.7 ppm were also observed, which indicate the presence of unsaturation in the esters present in biodiesel. The peak observed at 13.5 ppm was due to terminal carbon of methyl group while at 22–33.5 ppm the peak was due to methylene carbons present in the backbone of esters of biodiesel. The spectra of the algae biodiesel were observed to have similar peaks as present in the ^{13}C NMR (Fig. 5e) spectra of karanja biodiesel. The ^{13}C NMR spectra of the diesel (Fig. 5f) showed the group of peaks at 20–40 ppm from aliphatic carbons.

3.3.5. FTIR

The peaks or bands in the FTIR spectrum are due to the functional groups present in a particular sample. In the present study, mid FTIR region was selected to identify the functional groups in the particular sample. Fig. 6 shows the FTIR spectra of the dry algae biomass and deoiled biomass of the algae species. The region in the spectra from 3100 to 2800 cm^{-1} indicates the presence of lipids in the sample and is due to the symmetrical and asymmetrical stretching vibrations of –CH$_2$– groups. These –CH$_2$– groups form the backbone of the lipids and show the absorption particularly at 2927 and 2860 cm^{-1}. On comparing the intensity of the band in this region in the spectra for the algae biomass and deoiled biomass, the decrease in intensity was observed in the latter, which implies that lipids have been successfully extracted out from the biomass. Similar changes were observed on comparison of the spectra of pond water algae and *Spirulina* algae biomass and deoiled biomass. The FTIR spectra (Fig. 7a and b) of algae oil (pond water and *Spirulina* respectively) were compared with their respective biodiesel

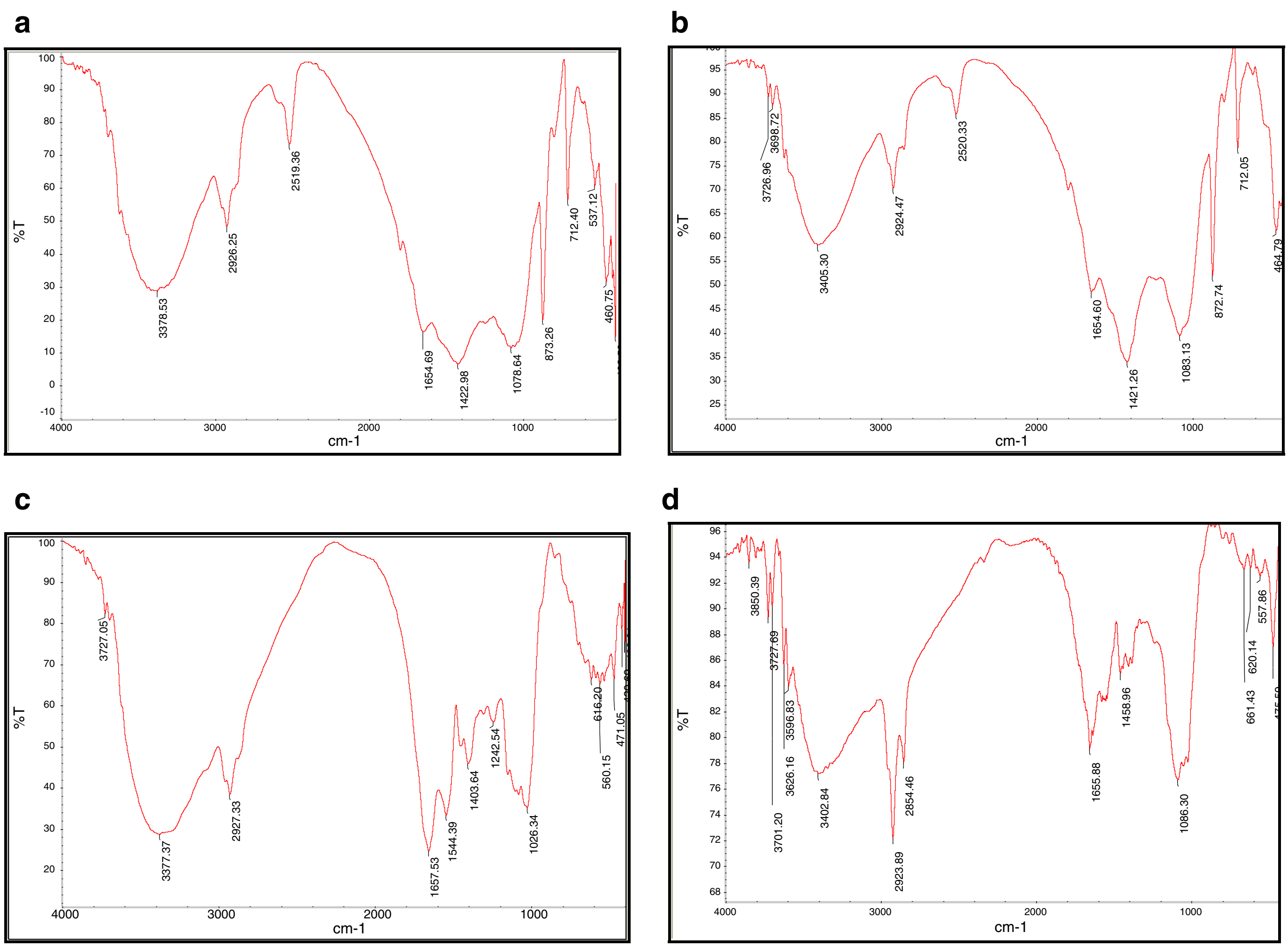

Fig. 6. FTIR spectra of (a) pond water algae biomass and (b) pond water algae deoiled biomass (c) *Spirulina* algae biomass (d) *Spirulina* algae deoiled biomass.

spectra (Fig. 7c and d), which showed the well absorbed regions of 3500–3000 cm^{-1}, 1800–1000 cm^{-1} and 800–700 cm^{-1}. The typical peaks at 2927 and 2860 cm^{-1} are due to symmetrical and asymmetrical stretching vibrations of $-CH_2-$ groups. The peaks at 3005 cm^{-1} due to double bond stretching and 1300–1100 cm^{-1} due to C–O bond (axial stretching) were also observed in the spectra of algae oil and biodiesel. Absorption peaks were also noticed at 722 cm^{-1} due to $-CH_2-$ bending out of the plane and at 1365–1377 cm^{-1} due to $-CH_3$ bond. Similar peaks were observed in the FTIR spectra of algae oil except a peak in the range of at 1437–1460 cm^{-1} in biodiesel spectra only, which is due to the methyl ester moiety and at 1743 cm^{-1} corresponding to the >C=O stretching. The presence of these peaks indicates the conversion of oil to biodiesel. Similar peaks were observed in the spectra of pond water algae and *Spirulina* algae (both for algae oil and biodiesel). The FTIR spectra of algae biodiesel was observed to have the similar bands as present in karanja biodiesel (Fig. 7e), which further strengthens the fact that algae biomass too has the potential for biodiesel production.

Comparing the FTIR spectra of diesel (Fig. 7f) with that of biodiesel, peak at 1743 cm^{-1} was not observed in the case of diesel, which is due to carbonyl group present only in biodiesel. Moreover, diesel is mainly composed of aliphatic hydrocarbon which shows absorption at 2924 cm^{-1} and 2853 cm^{-1}.

3.3.6. GC analysis

GC analysis (Fig. 8) was used to study the chemical composition of algae biodiesel produced from pond water algae and *Spirulina*. The major peaks were identified using the SupelcoTM 37 Component fame mix standard for both the biodiesel samples. The peaks in the chromatograms of biodiesel samples and the standard were compared and their

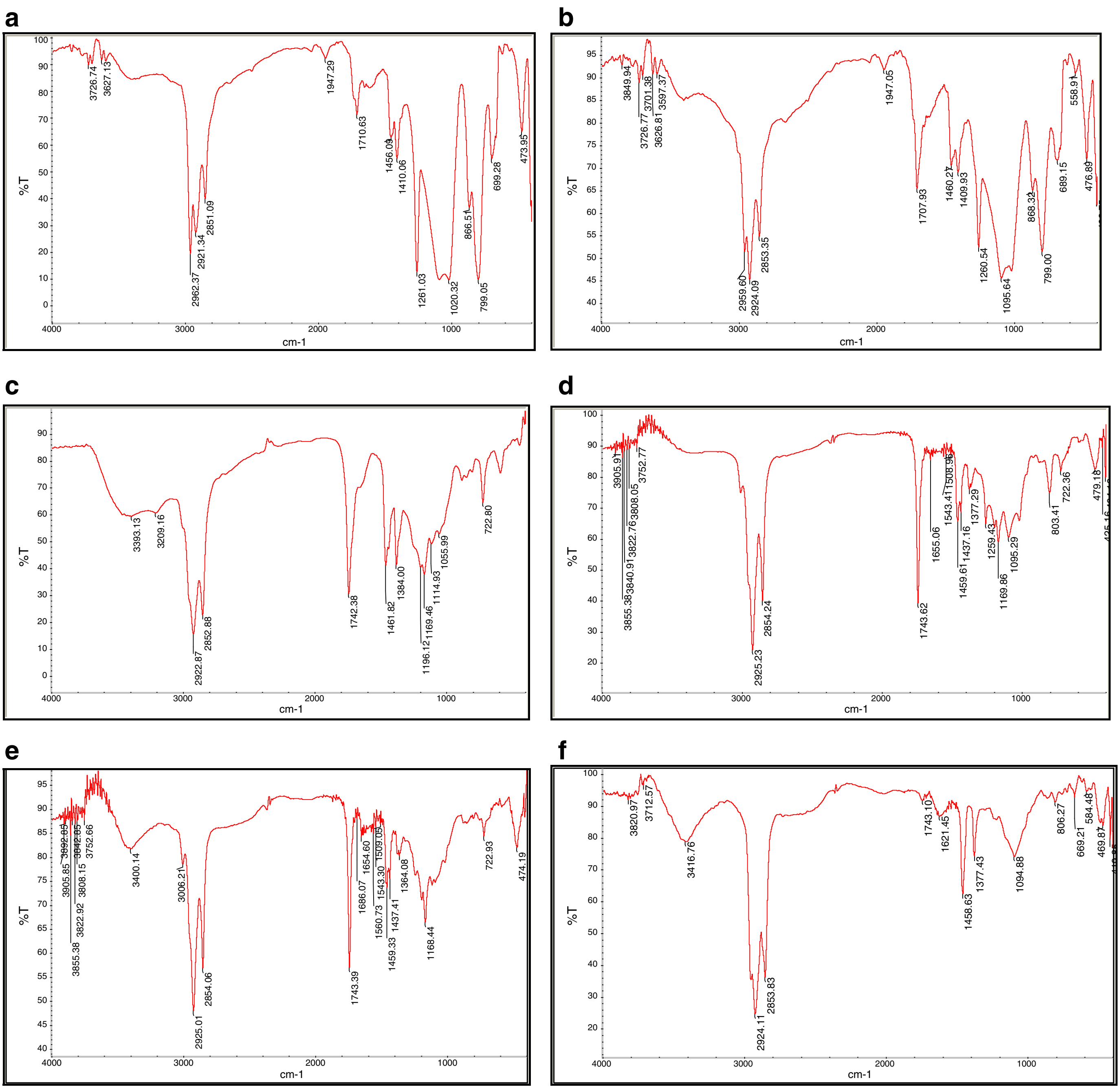

Fig. 7. FTIR spectra of (a) pond water algae oil, (b) *Spirulina* algae oil, (c) pond water algae biodiesel, (d) *Spirulina* algae biodiesel, (e) Karanja biodiesel, and (f) diesel.

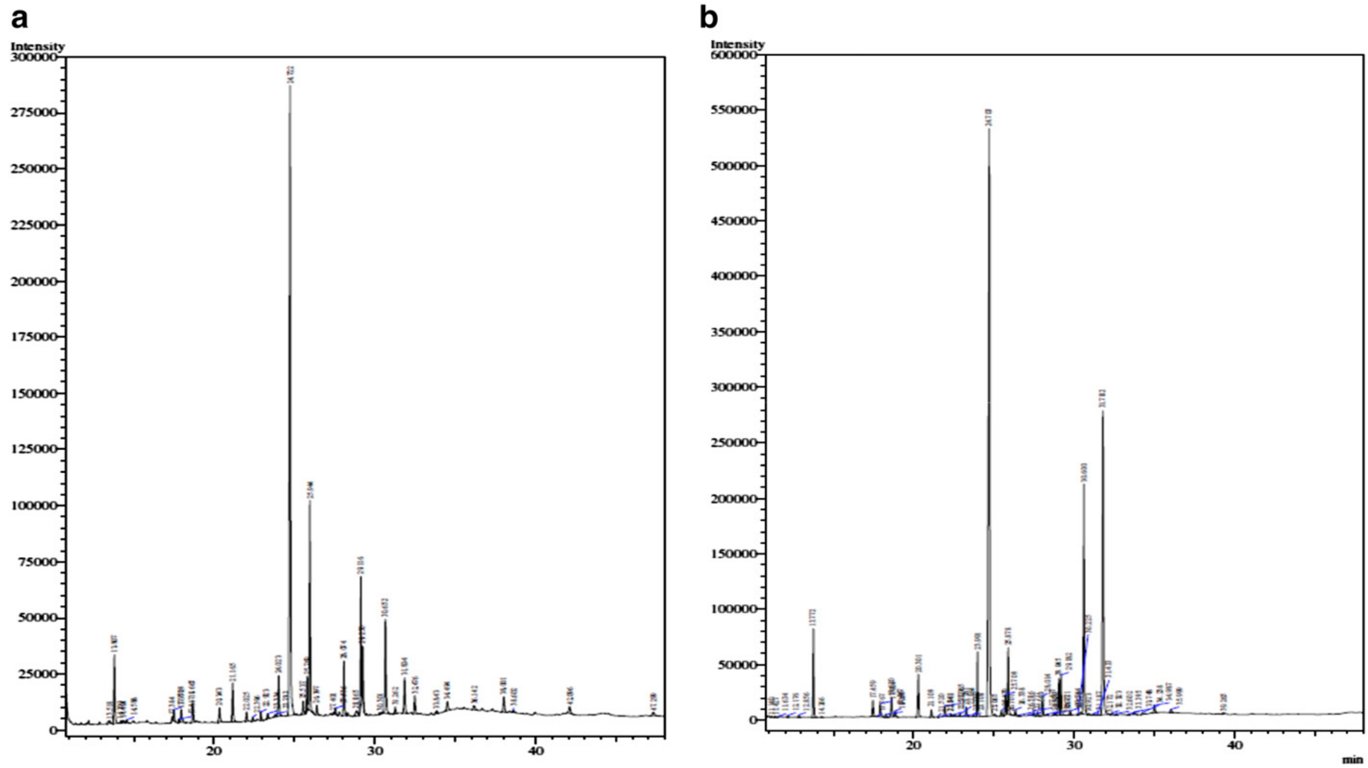

Fig. 8. GC analysis of (a) pond water algae biodiesel (b) *Spirulina* algae biodiesel.

respective retention time was used to identify and quantify the peaks. The analysis majorly showed the presence of five saturated (palmitic, caprylic, myristic, lauric, and stearic) and five unsaturated (linolenic, linoleic, oleic, palmitoleic, and pentadecanoic) methyl esters in both the algae biodiesel chromatograms. However, one extra peak of polyunsaturated fatty acid (Eicosapentaenoic) was observed in the case of pond water algae biodiesel. Table 3 shows that the composition of fatty acid methyl esters present in the pond water algae biodiesel and *Spirulina* biodiesel is the same with respect to the sum percentage of saturated and unsaturated fatty acid methyl esters. Palmitic acid methyl ester is the maximum in both the algae biodiesel. Pond water algae biodiesel is rich in oleic and palmitoleic methyl esters, whereas *Spirulina* is rich in linoleic and linolenic methyl esters. The fatty acid composition is also compared with that of tallow, karanja and palm biodiesel (Table 3). Tallow and palm biodiesel were observed to be rich in palmitic and oleic acid methyl ester, whereas karanja biodiesel was rich in oleic acid methyl ester.

3.3.7. Fuel properties

The fuel properties of *Spirulina* algae biodiesel and pond water algae biodiesel were determined and compared with diesel in Table 4. Most of the properties satisfy the standards prescribed by the American and European standards. The density of the biodiesel sample in the present study for *Spirulina* and pond water algae was 860 kg/m^3 and 870 kg/m^3 respectively, which satisfies the European standards. The density of the biodiesel obtained in the present study was found to be comparable with other varieties of algae biodiesel [32]. The density of biodiesel for algae biodiesel obtained in the present study was less as compared to density of biodiesel from karanja, jatropha, and waste cooking which is about 884 kg/m^3, 880 kg/m^3, and 883.5 kg/m^3 respectively [33–35].

Table 3
FAME$_s$ (fatty acid methyl esters) composition of biodiesel (a) Pond water algae (b) *Spirulina* algae (c) Tallow (d) Karanja (e) Palm.

S.No	FAME	Formula[a]	Structure[a]	wt.% of FAME in biodiesel				
				(a) Pond water algae	(b) *Spirulina*	(c) Tallow [29]	(d) Karanja [30]	(e) Palm [31]
1.	Caprylic	$C_8H_{16}O_2$	8:0	3.76	3.90	–	–	–
2.	Lauric	$C_{12}H_{24}O_2$	12:0	2.23	1.14	–		
3.	Myristic	$C_{14}H_{28}O_2$	14:0	3.33	2.52	5.40	–	–
4.	Cis-10-Pentadecanoic	$C_{15}H_{28}O_2$	15:1	2.26	3.07	–	–	–
5.	Palmitic	$C_{16}H_{32}O_2$	16:0	38.39	41.21	32.8	10.60	40.30
6.	Palmitolec	$C_{16}H_{30}O_2$	16:1	13.17	3.39	4.30	–	–
7.	Stearic	$C_{18}H_{36}O_2$	18:0	3.12	1	4.10	6.80	3.10
8.	Oleic	$C_{18}H_{34}O_2$	18:1	11.73	4.11	35.10	49.40	43.40
9.	Linoleic	$C_{18}H_{32}O_2$	18:2	5.53	12.64	15.70	19	13.20
10.	Linolenic	$C_{18}H_{30}O_2$	18:3	3.25	17.79	1.60	–	–
11.	Eicosapentaenoic	$C_{20}H_{30}O_2$	20:5	1.12	–	–	–	–

[a] Of corresponding fatty acid.

Table 4
Fuel properties of algae biodiesel and diesel.

S.No	Parameter	Spirulina biodiesel	Pond water algae biodiesel	Diesel [33]	ASTM D6751	EN 14214
1.	Density (kg/m^3)	860	872	831	–	860–900
2.	Viscosity (mm^2/s) at 40 °C	5.66	5.82	3.21	1.9– 6.0	3.5–5.0
3.	Specific gravity	0.865	0.878	–	0.88	–
4.	Acid number (mg KOH/g)	0.45	0.40	0.20	0.50 max	0.50 max
5.	Calorific value (MJ/kg)	41.36	40.80	42.232	–	-
6.	Pour point (°C)	−18	−16	−17	–	–
7.	Flash point (°C)	130	–	76	Min. 100–170	Min. 120
8.	Copper strip corrosion	1 (slight tarnish)	1	–	No. 3 max	No. 1 min

The viscosity of biodiesel from *Spirulina platensis* and pond water algae in the present study was found out to be 5.66 mm^2/s and 5.92 mm^2/s respectively which is lower as compared to biodiesel obtained from waste rapeseed oil and fish oil (6.35 mm^2/s and 7.2 mm^2/s respectively) [36,37]. The viscosity of biodiesel from *Nannochloropsis sp*. algae *is 5.76* mm^2/s [38]. Specific gravity of *Spirulina* biodiesel and pond water algae biodiesel was 0.865 and 0.878 respectively. Density and viscosity of particular a fuel affect the brake specific fuel consumption. The higher the density and viscosity, the higher will be the mass of fuel injected which will increase the brake specific fuel consumption. Moreover, higher values of density and viscosity lead to poor atomization and poor air fuel mixing which leads to poor combustion and ultimately decrease the efficiency of engine. The density and viscosity of conventional diesel is 831 kg/m^3 and 3.21 mm^2/s respectively [33]. These values are comparable to the density and viscosity values of algae biodiesel from *Spirulina* and pond water algae and can be easily form blends for running the engine.

Acid value is related to long term stability of biodiesel against corrosiveness. The lower the acid value, the better is the quality of biodiesel. The acid value for *Spirulina* and pond water algae biodiesel was determined to be 0.45 mg KOH/g and 0.4 mg KOH/g respectively. The acid value of diesel (0.2 mg KOH/g) is less [33] as compared to biodiesel due to presence of acidic components (esters) in the chemical structure of biodiesel. The acid values of beef tallow biodiesel and *Scenedesmus* algae biodiesel was found to be 0.495 mg KOH/g and 0.52 mg KOH/g respectively [39,32]. Calorific value also affects the brake specific fuel consumption because more fuel with lower calorific value is required to maintain the specified power. Calorific value of biodiesel from *Spirulina platensis* and pond water algae was found to be 41.36 MJ/kg and 40.8 MJ/kg respectively which are higher as compared to calorific value of 39.112 MJ/kg for karanja biodiesel [33]. The calorific value of biodiesel is lower than the diesel (42.232 MJ/kg) because of the presence of extra oxygen in the biodiesel [33]. Flash point of conventional diesel is about 76 °C [33] as compared to 130 °C of biodiesel from *Spirulina* algae biodiesel. The higher the flash point, the lower will be the chances of premature ignition. Pour point of a liquid fuel is the lowest temperature at which it loses its flow properties. Pour point of *Spirulina* algae biodiesel and pond water algae biodiesel was in the specified range laid down by the American and European standards. The copper corrosion for both the biodiesel fell into Class 1 which implies that engine parts will suffer less corrosion with time.

4. Conclusion

From the growth study of the algae species conducted in the present work, it can be concluded that *Chlorella* showed the maximum growth and higher growth rate based on the chlorophyll content and dry cell weight both in the presence and absence of BG 11 media. Pond water algae grow almost at the same rate as that of *Spirulina* algae. Comparing the results of proximate analysis, moisture content in *Chlorella* was higher as compared to *Spirulina* and pond water algae whereas the volatile matter and fixed carbon on dry-ash-free basis for all the three algae biomass were in the comparable range. Elemental composition of all the three algae species were also more or less the similar. The proximate and elemental composition of algae biodiesel was found comparable with that of karanja biodiesel which is already an established biodiesel. Thus pond water algae can be considered as a viable feedstock for biodiesel production as economic viability of biodiesel is dependent upon the price of feedstock used for biodiesel.

The single stage process of simultaneous extraction and transesterification result in higher biodiesel yield as compared to extraction followed by transesterification in two stages. The spectra of NMR and FTIR and gas chromatographic analysis data showed very clearly that algae biodiesel obtained from *Spirulina* and pond water algae biomass were composed of fatty acid methyl esters. Gas chromatographic analysis data also showed that algae biodiesel derived from *Spirulina* and pond water algae mainly composed of palmitic acid methyl ester. Pond water algae biodiesel has more percentage of oleic and palmitoleic acid methyl ester as compared to *Spirulina* biodiesel. On the other hand, *Spirulina* biodiesel has more linoleic and linolenic acid methyl ester as compared to biodiesel derived from pond water algae. Fuel properties of algae biodiesel show that biodiesel from *Spirulina* algae and pond water algae were comparable in quality with that of other conventional biodiesel. Based on the fatty acid composition and the fuel properties, it is concluded that biodiesel produced from *Spirulina* and pond water algae could be a good alternate to conventional diesel.

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