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Feasibility of Biodiesel production from *Chlorella Vulgaris*grown in flat plate photobioreactor under outdoor conditions

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Abstract: Microalgae biomass has the potential to provide different types of renewable transportation fuel such as biodiesel, bioethanol, bio-hydrogen, and biogas. Among them, microalgae based biodiesel is getting more attention now days. However, large-scale production has many challenges to satisfy the expected future demand for biodiesel produced from microalgae. This investigation aims to examine the feasibility of biodiesel from *Chlorella vulgaris* microalga grown at large scale under outdoor conditions. *Chlorella vulgaris* was grown in 60 liter Flat plate photobioreactor at batch and semi-continuous mode The results demonstrated that *Chlorella vulgaris* showed maximum biomass productivity (164.3 mg/L/d) with lipid content of 14.17% when two third culture was harvested, but lipid productivity was found maximum (23.92 g/L) when one half culture was harvested during semi-continuous mode. Fatty acid profile of *Chlorella vulgaris*grown in flat plate photobioreactor has shown abundance of fatty acids with carbonchain length of C16 and C18.Moreover, the fuel properties of biodiesel such as viscosity, density, Cetane number, saponification value,iodine value, and cold filter plugging point were found accordance with Indian standards IS 15607: 2005, Standard ASTMD6751, and European Standard (EN 14214).
Keywords : Microalgae, Flat plat photobioreactor, semi-continuous culture, lipid, Biodiesel, outdoor conditions.

Introduction

Increasing oil prices, depletion of natural resources, greenhouse emissions by combustion of fossil fuel and energy security forced researchers to search the alternative environmental friendly energy sources in the recent years[1, 2]. Biofuels such as biodiesel, ethanol and biogas are getting more attention to address above issues. Among them, biodiesel is a renewable, non-toxic, and biodegradable fuel which can be used in existing diesel engines without modifying the engines. Furthermore, it can be blended in at any ratio with petroleum diesel. Biodiesel can be produced from edible (soybean, rapeseed, mustard oil and canola oil etc.) and non-edible oil (Jatropha, Castor, Mahua, Polanga and Karanja oil etc). However, due to the limitation of available agriculture land for edible crops, it disturbs food chain; therefore, other sources of vegetable oil have to be developed as feedstock for biodiesel. In the present time, microalgae are being explored as a sustainable and renewable energy feedstock[3,4]. According to Chisti (2008), microalgae are able toproduce 20 times more biodiesel (on the basis of productive per unit area) than the best oil-seed crop such as plam[5]. Microalgae are microscopic photosynthetic organisms and found in both marine and fresh water environments. Unlike oil crops, the growth rate of microalgae is very high and some species double their biomass within 24 h [3]. Biomass doubling times during exponential growth are generally as short as 3.5 h. The lipid content of some microalgae

can be increased upto 80% (by weight of dry) [3]. Microalgae lipid content can be improved under nutrient starvation condition, such as nitrogen and phosphorous deficient condition. Furthermore, 1kg of microalgae is able to fix 1.8 kg of CO_2. Although microalgae shows a great potential for biodiesel production at lab scale, but commercial production of biodiesel from microalgae still have many challenges[3, 6, 7]. In this study, a green microalgal strain *Chlorella vulgaris* was selected and cultured in 60 litter capacity flat plate photobioreactor (FPP) at batch and semi-continuous mode under outdoor conditions. In addition, fuel properties of biodiesel derived from microalgae were also compared with ASTM D6751 and IS 15607: 2005 standards.

Experimental

Strain and pre- cultivation Condition

Pure cultures strain of *Chlorella vulgaris* was obtained from Vivekananda institute of algal technology (VIAT), Chennai (India); and maintained at agar slant periodically. The cultures were maintained in 250 ml Erlenmeyer flasks containing 100 ml BBM medium with initial pH of 6.8 and incubated under cool florescence light (~2500 lux) at 24°C (±1°C) with 16 :8 Light : Dark cycle in a photo bioreactor for 14 days.

Cultivation of microalgae in 60 litter flat plate photobioreactor at batch and semi-continuous mode

The photobioreactor used microalgae cultivation was 60 L FPPs (flat plate photobioreactor; 120 × 15 × 45 cm = length× thickness× height). The top opening of the photobioreactor was covered with a cap made of transparent acrylic plat. Aeration was provided by aquarium pump from 9:00 am to 6:00 pm. BG-11 medium was used as culture growth media. Generally, sodium nitrate was used as nitrogen source in BG-11 media but in this study sodium nitrate was replaced by urea. The composition of BG-11 growth media was as follows: Urea 250 mg L^{-1}, K_2HPO_4 40 mg $L^{-1}$$MgSO_4$-7H2O 75mg L^{-1}, $CaCl_2$-2H_2O 36 mg L^{-1},Citric acid 6 mg L^{-1}, Trace metal solution 1 ml L^{-1} (Trace metal solution: $FeC_6H_5O_7$-NH_4OH 6 g L^{-1}, Na_2EDTA 1 g L^{-1}, $MnCl_2$.4H_2O 1.81 g L^{-1}, $ZnSO_4$-7H_2O 0.222 g L^{-1}, Na_2 MoO_4-2H2O, 0.39 g L^{-1},$CuSO_4$-5H_2O 0.08 g L^{-1}, H_3BO_3 2.86 g L^{-1} . The experiment was conducted for 15 days at batch mode under outdoor conditions. The temperature variation of medium was between 20°C and 37°C and light intensity was about 1Klux during sunrise and sunset while the highest light intensity was about 84Klux during afternoon. Different growth parameters such as biomass concentration, lipid content, biomass productivity and lipid productivity were analyzed.

During semi-continuous mode, three experiments were carried out as follows: one third (growth phase a), half (growth phase b) and two third culture (growth phase c) harvesting at every 4th day. Total 6 harvests were done during each experiment. Dewatering of microalgae grown in Flat plate photobioreactor was performed using alum (as flocculent), followed by centrifugation.

Determination of optical density, dry weight concentration and lipid extraction

The optical density was determined at 680 nm in a UV-Visible spectrophotometer (thermo). The following correlation was used to convert the optical density OD680 into biomass concentration:

$$y=0.4032\times OD680 +0.0564 \qquad (R2=0.9624)\ldots(1.)$$

The dry weight concentration was determined to confirm the correlation. Samples with a volume of 10 ml were centrifuged (10 min per step at 800 rpm) and washed twice with distilled water. The samples were dried for 4-5 h at 105°C and weighed. Lipid was extracted using by folch extraction method[9].

Transesterification of Microalgal Lipid

In the transesterification, first lipid was extracted from 25 g dry microalgae by Chloroform: methanol (2:1) solvents by soxhlet method. The extracted lipid (1g) was transesterified using two step transesterification. In first step, the esterification was carried out using methanol to oil ratio 1:10 and 1.5% H_2SO_4 (v/v) at temperature of 60 °C for 2.5 h. after reduction of FFA less than 1% , transesterification was performed using 0.8 % potassium hydroxide (wt/vol.) as base catalyst and 1:20 lipid to methanol ratio (v/wt). The reaction was carried out into a 100 ml Round Bottom (RB) flask equipped with a reflux condenser, magnetic stirrer and thermometer. The temperature was maintained 65 °C for 3 h. After completion of reaction, the product was washed with hot water until the water layer reached pH7. Purified biodiesel was dried using sodium sulfate and followed by filtration using Whatman filter paper No.42. Purified biodiesel was analyzed by gas chromatograph.

Determination of fatty acid profile

FAMEs were examined by using a gas chromatograph (nucon 5785) with a flame ionization detector. Nitrogen was used as carrier gas with a flow rate of 1.2 ml /min. The injector and detector temperature were set at 240 °C and 260 °C, respectively. The oven temperature was initially held at 160º C and gradually raised to 240º C. Total run time was kept 50 min.Resulted analytes were identified and quantified with their authentic standards.

Determination of Physico –chemical properties of biodiesel

The biodiesel sample obtained at the lab scale experiments was in very less amount and not sufficient to analysis the fuel properties. Therefore, important fuel properties of biodiesel, that is Saponification value (*SV*), Iodine value (*IV*), Cetane number (*CN*) , including density, kinematic viscosity, oxidation stability and heating value [10,11,12].

Saponification value

$$\mathbf{SV} = \frac{\Sigma\,(560 \times N)}{Mw}$$

Iodine value

$$\mathbf{IV} = \frac{\Sigma\,(254 \times N \times D)}{Mw}$$

Viscosity η,

$$\ln(\eta_i) = -12.503 + 2.496.\ln(M_i) - 0.178.N$$

Density ρ,

$$\rho_i = 0.8463 + \frac{4.9}{M_i} + 0.0118.N$$

Calorific value, CV

$$CV_i = 46.19 - \frac{1794}{M_i} - o.21.N$$

Cetane number (CN)

$$CN_i = -7.8 + 0.302.M_i - 20.N$$

Where where ($\eta_i$is the kinematic viscosity of at 40 °C in mm^2/s; $\rho_i$is the density at 20 °C in g/cm3; and *CVi*is the calorific value and CNi is the cetane number in MJ/kg of *i*th FAME.

Oxidation stability, OS

$$\mathbf{OS} = \frac{117.9295}{z} + 2.5905$$

Where zis the content of linoleic and linolenic acids (wt%) ($0 < X < 100$); and *OS*is the oxidationstability in hours.

Result and Discussion

Cultivation of Chlorella vulgaris at batch and semi-continuous mode in 60 liter flat plate photobioreactor

In this study, *Chlorella vulgaris* were grown in a 60 L flat plate photobioreactor under outdoor conditions (figure 1). The experiments were carried out between February 2014 and April 2014. During the experiments, the daily (9:00 am–6:00 pm) temperature variation of medium was between 20°C and 37°C and light intensity was about 1Klux during sunrise and sunset while the highest light intensity was about 84Klux during day time. A constant aeration with an aeration rate about 8 liter/minute was provided by utilizing aquarium air pump. The results revealed that the microalgae grew well and achieved biomass concentration of

1.09 mg/L with 15.96% lipid content within 15 days. However, maximum biomass and lipid productivity (81.64 mg/L/d and 13.51mg/L/d) was found at 9th days (calculated from figure 2). Maximum

biomass productivity obtained in current study (81.64 mg/L/d) was higher than the biomass productivity of *C. vulgaris* (40 mg/L/d) and *C. emersonii* (41 mg /L/d) cultured in 230-L pumped tubular photobioreactor indoors reported by Scragg et al. (2002) and *C. emersonii* (58 mg/L/d) cultured in 60 liter FPP under outdoors[13]. However, biomass productivity observed in this study was lower than the biomass productivity of *Spirulinaplatensis*(4300 mg/L/d) reported by Hu et al. (1996). The fabulous productivity of *S. platensis* was achieved in FPPs with a light path of 2.6 cm[14]. In the current study, the light path of the photobioreactor was 15 cm, which could significantly reduce the light penetration in the culture, decreasing the light availability in the photobioreactor. As a result, the photosynthetic efficiency of microalgae might be significantly lower according to some literature[15,16]. Richmond et al. (2003) found that the photosynthetic efficiency of *Nanochloropsis sp.* cultures was nearly doubled when the light path length was reduced from 9 to 1 cm, the biomass concentration increased noticeably (3.9- 43.5 g/L). Whereas, the lipid productivity achieved in this study (13.51 mg/L/d) was similar to published data from other microorganisms grown under photoautotrophic cultivation outdoors, such as, 6.3–50.0 mg/L/d to 22.3 mg/L/d for *C. emersonii*[17] and can further be improved by shortening light path or increasing surface area/volume ratio of photobioreactor.An essential aspect of commercially successful microalgal culture is the ability to grow the microalgae in continuous or semi-continuous mode for long periods[18]. That can optimize the use of capital intensive culture systems and also decrease labor costs. The experiment was conducted only under the natural sunlight and no artificial light was provided. During semi-continuous mode, initially the microalgal cells were grown for 10 days at batch mode and then biomass was harvested at every 4 day intervals. A total of 6 harvests were done in each semi-continuous mode. At every harvest, the microalgal culture was replaced with same amount of nutrient medium. The experiment was conducted in three phase at i.e. growth phase a (one third harvesting), growth phase b (half biomass harvesting) and growth phase c (two third culture harvesting) as shown in figure 3a, 3b and 3c.The results demonstrate that *Chlorella vulgaris* cells grew well in Flat plate photobioreactor at semi- continuous cultivation mode under outdoor conditions. The average biomass productivity was observed between 115.21 mg/l/d and 164.37 mg/l/d and the highest biomass productivity was found 164.37 mg/l/d in growth phase C (table 1 and figure 3c). In addition, the average biomass productivity was lowest (121.65 mg/l/d) when one third culture was harvested (growth phase a).

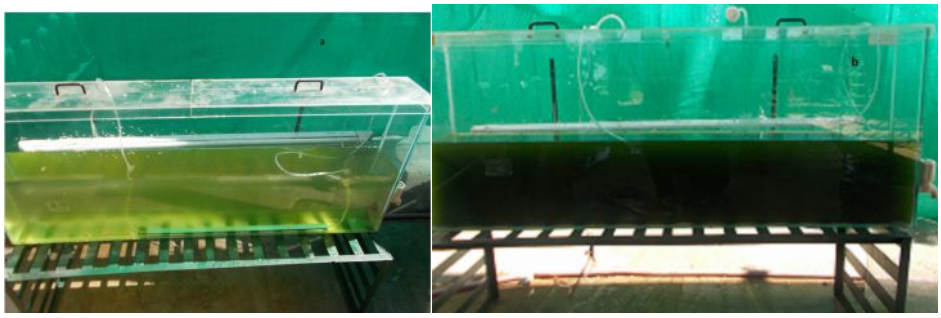

Figure 1.Flat plate photobioreactor used for algae cultivation (a) initial culture (b) culture after 15 days

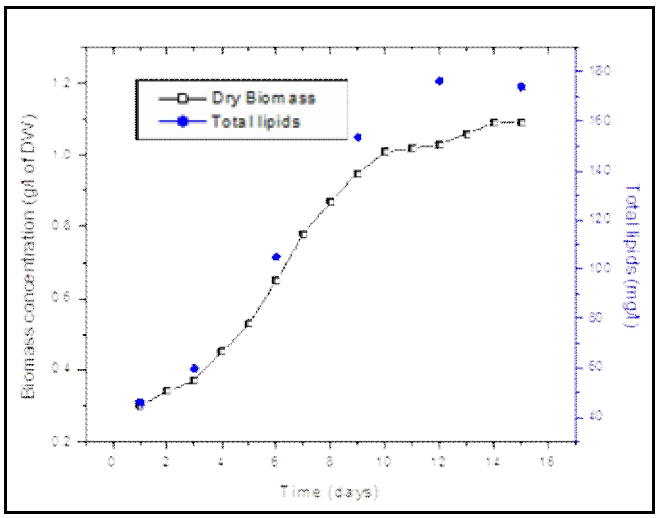

Figure 2.Biomass growth rate and total lipid content of microalgae in 60 liter Flat plate photobioreactor at batch mode

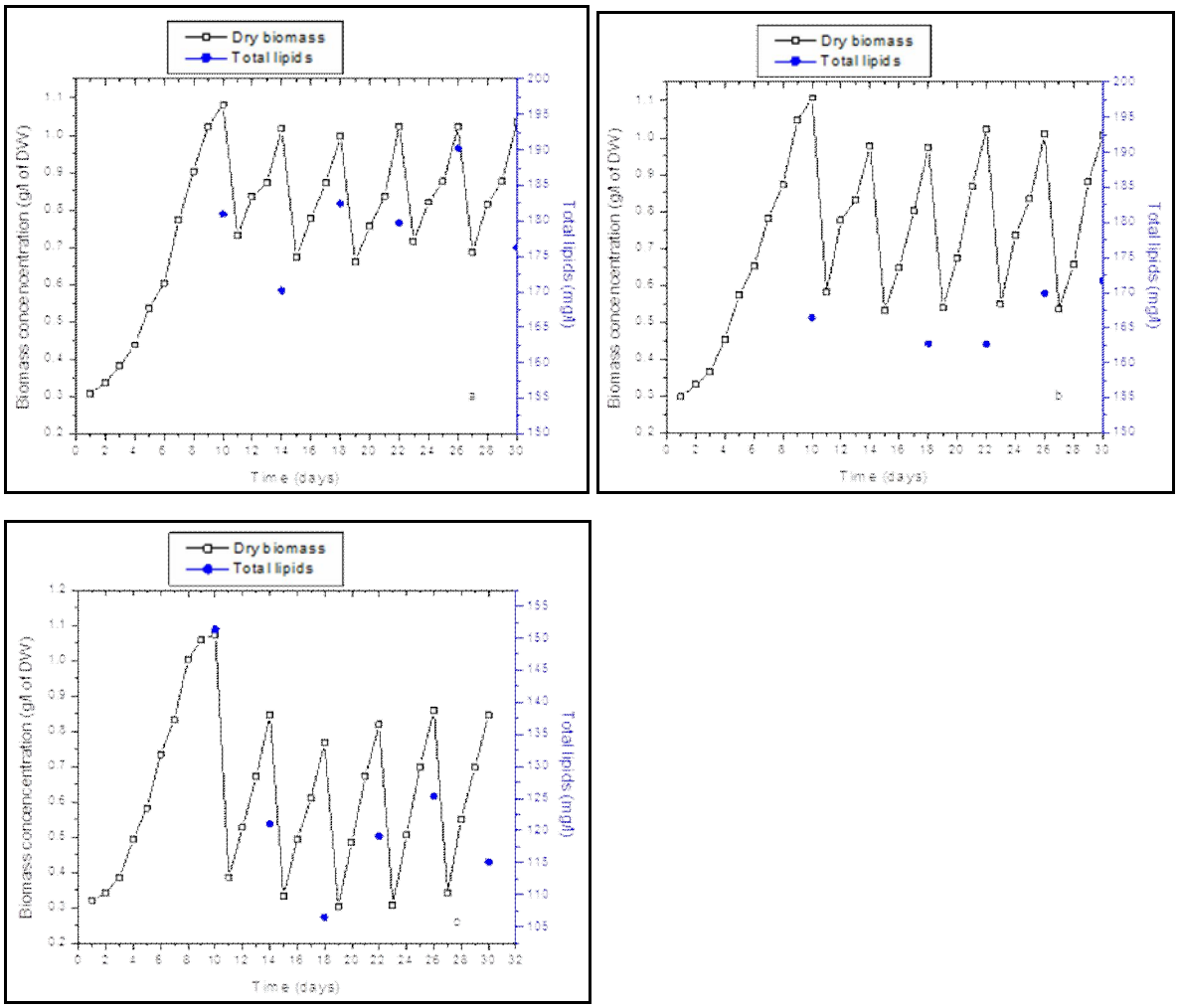

Figure 3.Biomass growth and total lipid yield of *Chlorella vulgaris* in three growth phases (a, b and c) of semi-continuous cultivation outdoors, a= one third culture concentration harvesting, b=half culture concentration harvesting, c=two third culture concentration harvesting.

Table1.Biomass growth and total lipid yield of *Chlorella vulgaris* in three growth phases (a, b and c) of semi-continuous cultivation outdoors, a= one third culture concentration harvesting, b=half culture concentration harvesting and c=two third culture concentration harvesting. Harvesting time for each growth phase was 4 days.

Growth phase	Biomass concentration (g/L)	BiomassProductivity (mg/L/d)	Lipid content(in %)	Lipid productivity (mg/L/d)
a (1/3th culture harvesting)	1.01 ± 0.01	115.21 ± 0.05	17.69 ± 0.73	20.40 ± 1.91
b (1/2 culture harvesting)	0.98 ± 0.01	145.93 ± 8.77	16.15 ± 0.88	23.92 ± 2.37
C (2/3th culture harvesting)	0.827 ± 0.03	164.3 ± 13.84	14.17 ± 0.38	22.99 ± 1.87

The outcomes of this study demonstrate that it is feasible to culture *Chlorella vulgaris* for a long period under outdoor conditions. In case of lipid production, it was observed that lipid content and yield was found highest (17.64%, 179.97 mg/l) in growth phase a. While the highest lipid productivity obtained as 23.92 mg/l/d in growth phase b. However, in growth phase C, the biomass productivity was supreme (164.3 mg/l/d) than other growth phases, but total lipid productivity (22.99 mg/l/d) was found less than growth phase b (table 1). These results show that *Chlorella vulgaris* achieved maximum lipid productivity when half culture harvested at 4th day during semi-continuous mode under outdoors (growth phase b).

Determination of Lipid extraction, biodiesel yield and fatty acid composition

Microalgae biomass was harvested using 250 mg/L alum as a flocculants, followed by filtration and solar drying. 80.21 % biomass was obtained by this harvesting technology. This dried biomass was used for oil extraction by soxhlet method and extraction efficiency was found to be 16.40%. Extracted lipid was re-dissolved in n-hexane and distilled to separate hexane from lipid. This process was repeated 3-4 times to get maximum lipids. This purified lipid was transesterified at 65 ^{o}C for 3 hour. This results in 74.99% of biodiesel yield. Fatty acid methyl ester (FAME) composition of biodiesel was shown in figure 4. It was found that saturated, mono saturated and poly saturated FAME composition of biodiesel was 39.65%, 20.58% and 37.68% respectively. Palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and α-linolenic (C18:3) acid shared 27.36%, 19.52%, 27.47% and 10.21% respectively of total FAME. The presence of high amount of saturated FAME results in higher stability but poor cold flow properties while presence of more polyunsaturated FAME results in poor stability and good cold flow properties. In this study FAME composition have an appropriate ratio of saturated and unsaturated esters which lead to best fuel properties of biodiesel.

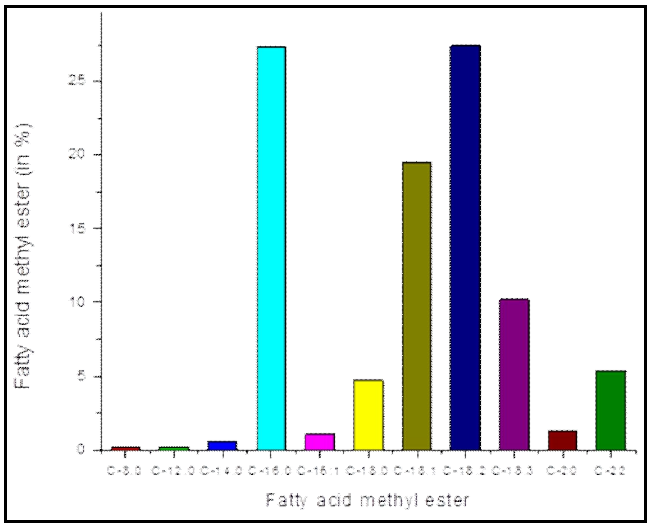

Figure 4.Biodiesel composition of *Chlorella vulgaris* microalga

Fuel properties of microalgae biodiesel

Fuel properties are highly dependent on FAME composition. In this study, fuel properties were calculated on the basis of FAME composition. Iodine and saponification value of microalgae biodiesel was found to be 94.09 and 191.56. CN is one of the most important indicators for determining combustion behaviour of diesel. The CN of a fuel is related to the ignition delay time (the time between injection and ignition). The shorter the ignition delay time, the higher the CN, and *vice versa*. According to the ASTM D6751-02 and IS: 15607- 05 standard for biodiesel, the minimum CN should be 47.0 and 51.0 respectively. Here CN of *Chlorella vulgaris* biodiesel was observed as 56.01. The examined oxidation stability of *Chlorella vulgaris* biodiesel was 5.71 which is slightly lower IS: 15607- 05 standards (minimum 6 hour) but follow ASTM D 6751 (minimum 3 hour).Cold filter plugging point (CFPP) values of biodiesel CFPP is a filterability test for cooled fuels already containing some solids. CFPP of the biodiesel was found to be -16.14 ^{o}C (Table 2). The viscosity and density of Chlorella vulgaris biodiesel was observed 4.58 Cstand .858 g/cm^3 which meets ASTM standards. The calorific value of biodiesel was examined as 38.85 MJ/kg.

Table 2.Fuel properties of biodiesel derived from *Chlorella vulgaris*

Fuel properties	unit	Chlorella vulgaris biodiesel	ASTM D 6751	BIS IS 15607- 05
Iodine value	g /100 g	94.09	-	-
Saponification value	mg/g	191.56	-	-
Cetane no.		56.01	47 min	51 min
Viscosity	Cst at 40^{o}C	4.21	1.9-6.0	2.5 - 6.0
Density	g/cm^3 at 15 ^{o}C	0.858	-	860-890
Calorific value	MJ/kg	38.85	-	-
Oxidation stability	Hour	5.71	3 min	6 min
Cold filter plugging point (CFPP)	^{o}C	-16.14	-	-
Acid value	mg KOH/g	.49	0.5	0.5

Conclusion

The results revealed that cultivation of *Chlorella vulgaris* in flat plate photobioreactor under outdoor condition has a great potential for large scale biofuel production. Fatty acid profile of microalga grown in flat plate photobioreactor has shown abundant of fatty acids with carbon chain length of C16 and C18. Various biodiesel properties such as Cetane number, iodine value, saponification value, viscosity and density were found to be in accordance with ASTM 6751-15 and IS: 15607- 05 which makes *Chlorella vulgaris* as a good raw material for biodiesel production.

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