Mass culture of marine microalgae *Chlorella vulgaris* (NIOT-74) and production of biodiesel

G. Dharani*, D. Magesh Peter, J. T. Mary Leema, T. S. Kumar, K. Thirupathi, A. Josephine, R. Kirubagaran and M. A. Atmanand

National Institute of Ocean Technology, Ministry of Earth Sciences, Chennai 600 100, India

Biodiesel production using marine microalgae as an alternate fuel source is receiving international attention in view of its economic and environmental advantages. The present study evaluated the feasibility of biodiesel production from the marine microalgae; *Chlorella vulgaris* (NIOT-74). Outdoor mass cultures of marine microalgae were done in different photobioreactors and raceways with marine *C. vulgaris* (NIOT-74) as a model organism. The study demonstrated the feasibility of producing biodiesel and provided an evaluation of the physico-chemical properties of biodiesel (B100) and blend (B10) according to ASTM standards. A cost-effective electroflocculation method with 90.12% harvesting efficiency was developed and tested. The biodiesel produced from *C. vulgaris* (NIOT-74) was tested in two-stroke and four-stroke engines and was also used to test drive a vehicle.

Keywords: Biodiesel, *Chlorella vulgaris*, fuel properties, photobioreactors.

Introduction

INDIA imports about 219.15 MT of petroleum, valued at US$ 90 billion (Rs 5.65 lakh crore) of which the automobile sector alone is reported to consume 70% of the total petroleum diesel stock[1]. India has a unique pattern of petroleum diesel consumption with five times more diesel consumption than gasoline in contrast to worldwide consumption standards of more gasoline than petroleum diesel. During the last two decades, Indian diesel consumption has increased several folds from 5 (1970–71) to 45 million metric tonnes (2006–07)[2]. This necessitates an urgent need for an alternative to petroleum diesel fuel for India[2]. Research activities have been focused on developing alternate fuels so that the huge cost involved in the import of petroleum diesel could be curtailed. Biodiesel is produced mostly from vegetable oil (edible and non-edible oil) or animal fats[3]. This is a major challenge to our food security as the potential market for biodiesel surpasses available plant and animal oil production[4]. An ideal feedstock is a fuel source with low green-house gas emissions and little or no competition with food production[5]. One such alternative is marine microalgae. Algal biodiesel performs as efficient as petroleum diesel with no sulphur and minimal emissions of particulate matter, CO, hydrocarbons, and SO_x (ref. 6). To translate biodiesel production from marine microalgae to a commercial reality, multifaceted research and development is essential starting from isolating and identifying suitable strains, to optimizing different stages of mass scale operations for biomass production, harvesting, and lipid extraction[2]. For any commercial venture on marine microalgae, it is pertinent to evaluate the performance of the strain under outdoor conditions[7]. The cost of marine microalgal biomass production can be brought down considerably by using natural sunlight and outdoor cultivation systems[8]. India being a tropical country has the advantage of sunlight to take up outdoor marine microalgal culture in an effective way to reduce cost of microalgal biodiesel production[9]. Majority of the studies on biodiesel production from marine microalgae are restricted to indoor cultures due to the difficulties in maintaining the outdoor cultures contamination free in the ever-changing weather[7]. Hence, the study attempted to investigate the growth performance of marine microalgae in different custom-designed outdoor photobioreactors under phototrophic and mixotrophic conditions. The opportunities and constraints for biodiesel production from marine microalgae were also evaluated.

Materials and method

Strain and culture conditions

Of the 200 marine microalgal strains screened from NIOT (National Institute of Ocean Technology) culture collections the best performing strain, *Chlorella vulgaris* (NIOT-74; NCBI accession number – JF894249) was used for all the experiments in this study. Cultures (500 ml) were grown aseptically in the laboratory at 25 ± 1°C, light intensity of 120 μmol photon s^{-1} m^{-2} (provided by cool-white fluorescence tubes) and a 16:8 h dark : light photoperiod. Sterilized natural seawater enriched with $f/2$ medium[10] (1 ml/l of seawater) in 1000 ml Erlenmeyer flask was used as culture medium for the indoor cultures.

*For correspondence. (e-mail: dhara@niot.res.in)

Figure 1. Marine *Chlorella vulgaris* (NIOT-74) cultured in: ***a***, continuous flow bubble column photobioreactor; ***b***, tubular photobioreactor; ***c***, harvested biomass under outdoor conditions.

Table 1. Nutrient composition of custom designed medium used for marine *C. vulgaris* (NIOT-74) outdoor cultivation systems

Nutrient	Phototrophic (g l^{-1})	Mixotrophic (g l^{-1})
Urea	1.0	1.0
Phosphate	0.11	0.11
$FeCl_3$ + EDTA	0.020	0.020
Sodium acetate	Nil	0.5

Outdoor microalgal cultivation experiments

The outdoor marine microalgae cultivation experiments were carried out in the outdoor marine microalgal culture facility of National Institute of Ocean Technology (NIOT), Chennai, India. This study was carried out from February to April under natural temperature and light conditions at the outdoor culture facility of NIOT.

Outdoor mass culture experiments

Bubble column photobioreactors used in the experiments were carried out in 320 litres capacity with a working volume of 300 litres. Open raceway pond experiments were performed in three FRP raceway systems, each with 2.5 tonne capacity and a working volume of 2.0 tonne capacity. The mixing and circulation of the culture medium was accomplished by a solar powered paddle wheel made of galvanized steel fixed to an axis. A transparent acrylic continuous flow bubble column photobioreactor of 1500 litre capacity was also developed (Figure 1 *a*). Air lift mechanism was employed in this system to achieve continuous flow rate of 300 l h^{-1}. Additionally, a tubular photobioreactor made of transparent acrylic 330 mm diameter and 2000 litre capacity with process control was also developed for maintaining temperature and pH (Figure 1 *b*). Pre-filtered, pre-chlorinated and de-chlorinated seawater, filtered through 0.2 µm size filter was used for the culture process with custom designed media (Table 1) utilizing urea as nitrogen source. The experimental cultures were inoculated with 10% of *C. vulgaris* culture in exponential phase, with an initial biomass concentration of 0.2 g l^{-1} from indoor cultures. The culture was done in triplicate and repeated thrice. Experiments were run for 11 days. Mass culture experiments were done under phototrophic and mixotrophic conditions. Loss through evaporation was compensated by the addition of filtered seawater.

Microalgal growth measurement

Samples (100 ml) were collected every day and growth was monitored turbidometrically by measuring the optical density (OD) at 540 nm using a spectrophotometer (Unicam UV 300, USA) and converted to biomass concentration using appropriate calibration curves. The biomass dry cell weight, maximum specific growth rate, doubling time, biomass productivity were determined following the method of Zhu and Lee[11].

Measurement of climatic conditions

Algal samples were collected every day at 9.00 a.m. Light intensity was measured using a lux meter and culture temperature was recorded at 9:00, 12:00 and 17:00 hours every day using a temperature probe (Hydrolab, Germany).

Concentration and harvesting

Harvesting by electroflocculation. Experiments were done for harvesting the cultured marine *C. vulgaris* (NIOT-74) using electroflocculation. A three phase direct current (DC-24V, 52 A) power supply system was developed and patented for electroflocculation of *C. vulgaris* (NIOT-74) using aluminium electrodes. The electroflocculation system was set in 300 litre acrylic tanks for 15 min and the flocculated algae were allowed to settle for 30 min. The algal culture used for elctroflocculation had a pH of 7.56 and temperature of 31.7°C. All the tests were done in triplicates. The effectiveness of flocculation was determined by the recovery efficiency (RE) which is the ratio of recovered biomass to the total biomass. The recovery efficiency was determined according to Lee *et al.*[12].

Total lipid content estimation

Lipid was extracted from lyophilized (100 mg) *C. vulgaris* (NIOT-74) algal cells at stationary phase using a

Table 2. Kinetic growth parameters of marine *C. vulgaris* (NIOT-74) grown in different outdoor cultivation systems under phototrophic and mixotrophic conditions

Cultivation system	Specific growth rate μ_{max} (day^{-1})	Doubling time t_d (days)	Biomass productivity ($P_{biomass}$) (g l^{-1} day^{-1}) (N = 18)	Final biomass concentration (mg g^{-1}) (N = 24)
Raceway	0.17 ± 0.01	4.08	0.02	0.34
BPR	0.18 ± 0.007	3.85	0.05	0.60
CFBPR	0.13 ± 0 .006	5.33	0.01	0.18
TPR	0.13 ± 0.007	5.33	0.01	0.18
Under mixotrophic conditions				
Raceway	0.30 ± 0.01	2.31	0.21 ± 0.015	2.01 ± 0.19
BPR	0.31 ± 0.007	2.23	0.32 ± 0.020	3.03 ± 0.24
CFBPR	0.25 ± 0.006	2.77	0.09 ± 0.004	0.96 ± 0.05
TPR	0.17 ± 0.007	4.07	0.02 ± 0.001	0.28 ± 0.01

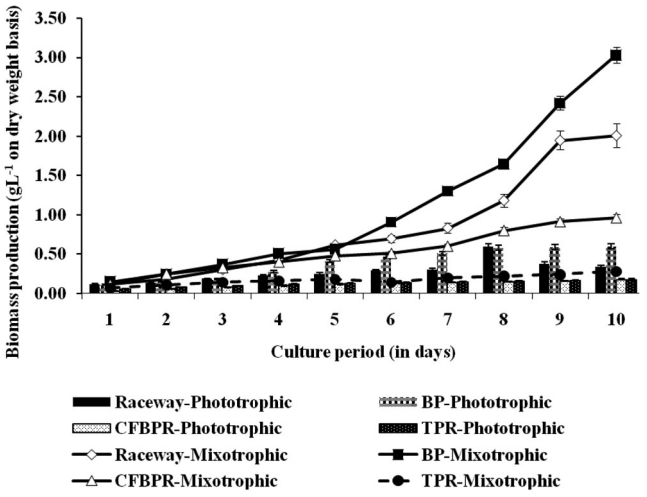

Figure 2. Growth of marine *C. vulgaris* (NIOT-74) in different culture systems under phototrophic and mixotrophic conditions.

solvent-based method modified from Folch *et al.*[13]. The total lipid was quantified gravimetrically. Each treatment was done in triplicate.

Fatty acid (FAME) determination

The fatty acid extracted was converted to methyl esters by transesterification of lipid following the method of Kashiwagi *et al.*[14].

Fourier transform infrared (FTIR) spectroscopy

For FTIR spectroscopy, 0.5 ml of algal biodiesel (B100), blend (B10) and petroleum diesel were used. FTIR spectra were collected using a Shimadzu IR affinity 1 model FTIR spectrometer in the ATR (attenuated total reflection) mode using lab solutions FTIR software. Spectra were collected over the wave number range 4000–600 cm^{-1}. Each sample was analysed in triplicate. Spectra were baseline corrected using the automatic baseline correction algorithm.

Biodiesel-internal combustion engine test

Biodiesel (B10 and B100) was tested in a four-stroke, single cylinder vertical air-cooled high-speed diesel engine fitted with data acquisition system (model: Kirloskar oil engine TAF 1) and the test parameters were monitored using AVL INDIMICRA 602-T10602A, software version V2.5. The engine performance was tested for its emission and combustion characteristics at various load levels like 25%, 50%, 75% and 100% with respect to biodiesel.

Statistical analysis

Statistical analyses were performed using SPSS version 19.0 software. The data was analysed using one-way analysis of variance (ANOVA), followed by post-hoc test of Tukeys H.S.D.

Results and discussion

Outdoor cultivation

The outdoor growth performance of *C. vulgaris* (NIOT-74) in different cultivation systems is shown in Figure 2. As indicated in Table 2, the maximum specific growth rate (μ_{max}) at the exponential phase was high (0.18 ± 0.007 day^{-1}) in bubble column photobioreactor (BPR) followed by raceway (0.17 ± 0.01 day^{-1}). The biomass productivity also showed a similar trend (Table 2). The specific growth rate of cultures grown in continuous flow bubble column photobioreactor (CFBPR) and tubular photobioreactor (TPR) was lower (Table 2). This consequently resulted in shorter doubling time (3.85 and 4.08 days) for *C. vulgaris* grown in BPR and raceway. The specific growth rate (0.17 day^{-1}) obtained for outdoor cultures under phototrophic conditions was higher than that reported for *C. ellipsoidea* (0.145 day^{-1}) under outdoor conditions[7].

To evaluate the feasibility of mass cultivation under mixotrophic condition, *C. vulgaris* (NIOT-74) was grown

in culture medium containing sodium acetate as carbon source in different culture systems. After a lag phase of one day *C. vulgaris* grew rapidly in different culture systems under mixotrophic conditions (Figure 2). As shown in Table 2 and Figure 2, the highest specific growth rate (0.31 g day^{-1}), final biomass concentration (3.03 g l^{-1}) and biomass productivity 320 mg l^{-1} day^{-1} were noticed in *C. vulgaris* (NIOT-74) grown in BPR. Lowest doubling time (2.23 days) was also observed in BPR. The specific growth rate (0.30 g day^{-1}), final biomass concentration (2.01 g l^{-1}) and biomass productivity (0.21 g l^{-1} day^{-1}) growth in raceway were very close to BPR (Figure 2; Table 2). The growth in CFBPR and TPR was significantly lower than that obtained in other two systems ($P < 0.05$). Notably, the biomass of 3.03 g l^{-1} achieved under outdoor condition was higher than that reported for *C. vulgaris* and *C. ellipsoidea*[15,16]. A biomass of 3 g l^{-1} is advocated for achieving economically viable production cost[8]. In this sense the biomass production of 3.03 g l^{-1} obtained under highly fluctuating temperature and light intensity seems to provide high prospects for industrial production.

Outdoor cultivation conditions and its impact on algal growth

The fluctuations of cultivation temperature during the experimental period in the different cultivation systems are depicted in Figure 3. It is evident that the maximum temperature during the culture period reached as high as 38°C. Notably, the *C. vulgaris* (NIOT-74) cultures were able to achieve biomass productivity of 0.76 g l^{-1} day^{-1} at this temperature. In line with the present study, Zhou *et al.*[17] have reported biomass productivity of 0.16 g l^{-1} day^{-1} for *C. pyrenoidosa* cultured under outdoor conditions at a maximum temperature of 35°C.

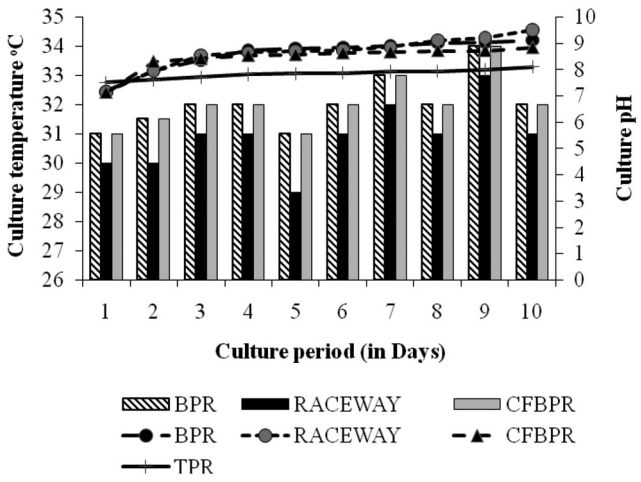

Figure 3. Average culture temperature and pH during culture period in different culture systems.

At the beginning of the experiment the pH in the cultivation systems of *C. vulgaris* was 7.11 (Figure 3). The pH gradually increased with growth of the algae and reached 9.2 at the end of the experiment. The increase in pH has been postulated to be related to increased photosynthetic activity[18] with concomitant increase in CO_2 consumption which ultimately lead to depletion of CO_2 and a shift in pH from acidic to alkaline[18].

Harvesting and dewatering of marine microalgae

Harvesting is one of the key processes that determine the economic feasibility of algal biodiesel production. In this study, dewatering was done using electroflocculation. After 15 min of continuous electric discharge the cells were completely separated as can be noticed in the thick green flocs observed and the biomass was totally separated from the culture medium. The relative efficiency of electroflocculation was 90.12 ± 2.19% for *C. vulgaris* (NIOT-74). The power consumption of the electroflocculation process was around 0.32 kWh/kg algal biomass. In line with the present findings, Ramos *et al.*[19] have also reported lower harvesting time (15 min) for electroflocculation of *Phaeodactylumcornatum* than centrifugation. Lower biomass yield and longer operation time make centrifugation less cost effective and higher energy consuming method[19]. To reduce the energy further, a solar powered electroflocculation was also developed.

Lipid content

The lipid content of *C. vulgaris* grown in different cultivation systems under outdoor conditions is shown in Figure 4. Maximum lipid content (19.76% as total lipid) was observed in *C. vulgaris* grown in raceway under mixotrophic condition. *C. vulgaris* grown in BPR had a lipid content of 16.37%. Nevertheless, under phototrophic conditions the lipid content in raceway and BPR was

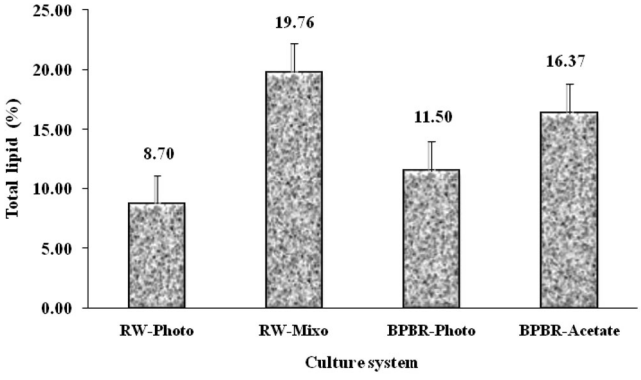

Figure 4. Total lipid (as percentage) of marine *C. vulgaris* (NIOT-74) biomass cultured in raceway (RW) and bubble column photobioreactor (BPBR) under phototrophic and mixotrophic conditions.

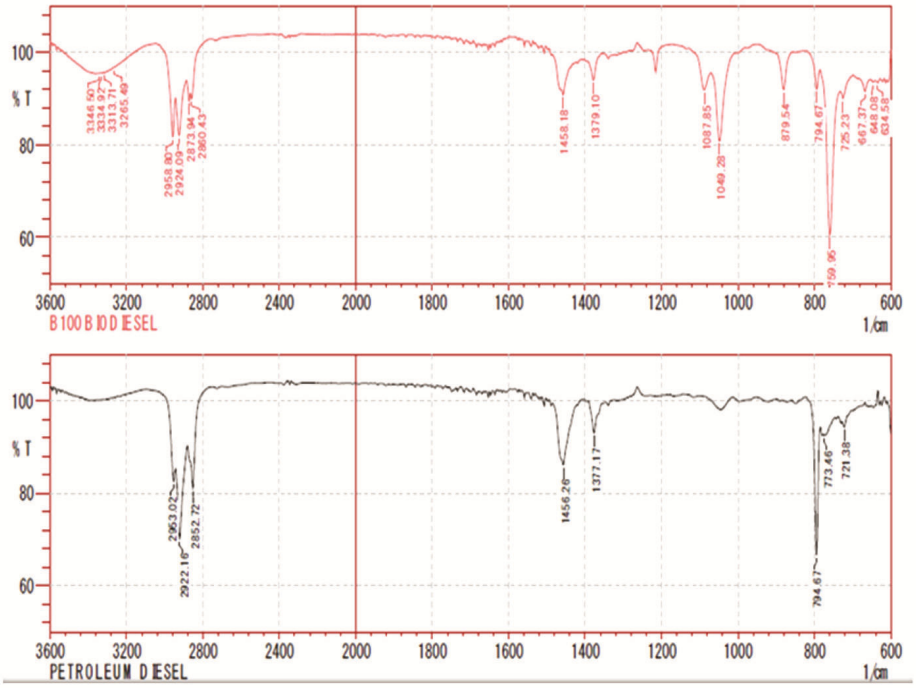

Figure 5. FTIR spectra showing biodiesel (red line) and petrodiesel (black lines).

Table 3. Fatty acid methyl ester (FAME) of *C. vulgaris* (NIOT-74) grown in outdoor in raceway

Fatty acid profile	Chemical formula	Fatty acid concentration (g 100 g^{-1})
Myristic	C14:0	11.7
Myristoleic acid	C14:1	0.13
Palmitic acid	C16:0	45.54
Palmitoleic acid	C16:1	31.10
Heptadecanoic acid	C17:1	0.67
Oleic acid	C18:1	6.68
Linoleic acid	C18:2	2.75
Linolenic acid	C18:3	0.05
Arachidic acid	C20:4	0.47
Saturated fatty acid		58.37
Unsaturated fatty acids		40.809

lower (8.70 and 11–50%). The lipid content grown in TPR and CFBPR was low under phototrophic and mixotrophic conditions. The greater fluctuations in temperature and light intensity under outdoor conditions must have caused this lower lipid content[7].

FAME composition

FAME composition of the microalgae has a direct bearing on the biodiesel fuel properties[20]. Thus, FAME composition determines the suitability of a microalga for biodiesel production. The dominant fatty acids in *C. vulgaris* were palmitic acid (C16:0) – 45.54%; palmitoleic acid (C16:1) – 31.10%; myristic acid (C14:0) – 11.70%; oleic acid (18:1) – 6.68%; linoleic acid (C18:2) – 2.75%; linolenic acid (C18:3) – 0.05% and arachidic acid (C20:4) – 0.47%. Saturated fatty acids play a pivotal role in determination of fuel properties. Cetane number, one of the crucial fuel properties increases with the increase in percentage of saturated fatty acids. Notably, *C. vulgaris* had higher saturated fatty acid content (58.39%). The fuel properties cloud point, pour point and density are affected by the percentage of unsaturated fatty acids. Hence an ideal candidate for biodiesel production should have optimal percentage of both saturated and unsaturated fatty acids[20]. As indicated in Table 4, *C. vulgaris* had 58.39% of saturated fatty acids and 38.01% of unsaturated fatty acids.

FTIR spectrometer analysis of biodiesel

The FTIR spectra of petroleum diesel and biodiesel (B100 produced from *C. vulgaris*) are shown in Figure 5. Both petroleum diesel and biodiesel showed similar characteristic bands of C–H stretching vibration corresponding to the presence of functional group alkane in the region 2850–3000 cm^{-1} and 1350–1480 cm^{-1}. Asymmetric and symmetric vibration modes of methyl groups were observed around at 2922 cm^{-1} for petroleum diesel and 2924 cm^{-1} for biodiesel. Nonetheless, the bands characteristic of biodiesel corresponding to O–CH_2–C asymmetrical stretching were present in the fingerprint region (900–1100 cm^{-1}) of B100 biodiesel and absent in the petroleum diesel. This further substantiates the

Table 4. Physico-chemical properties of *C. vulgaris* biodiesel blend (B10) compared to ASTM standard specifications

Parameter	B-100 biodiesel	ASTM limits
Acid number, ASTM D664	0.35 mg KOH/g	0.50 max. mg KOH/g
Observed flash point, ASTM D93	40.0°C	38°C minimum
Cloud point, ASTM D2500	3.0°C	–20.0°C
Sediment and water, ASTM D2709	1 ppm	500 ppm
Distillation of petroleum product at atmospheric pressure 90% recovered at room temperature, ASTM D86	68.0°C	360°C maximum
Kinematic viscosity @40°C, ASTM D445	1.45 mm^2/sec	1.9–6.0 mm^2/sec
Rams bottom carbon residue, ASTM D524	0.09%	0.35% mass maximum
Ash %, ASTM D482	0.001%	0.01% wt maximum
Sulphur content, ASTM D5453	0.01%	0.05% mass maximum
Copper strip corrosion @100°C for 3 days, ASTM D130	1 (a) rating	No. 3 rating maximum
Cetane index, ASTM D976	35	40 rating
Aromaticity, ASTM D1319	23.58%	35%, maximum
Wear scar diameter at 60°C, ASTM D6079	1.1 mm	0.45 mm maximum
Oxidative stability, EN14112	6 h	6 h minimum
Cetane number, ASTM D 613	56.4	47 minimum

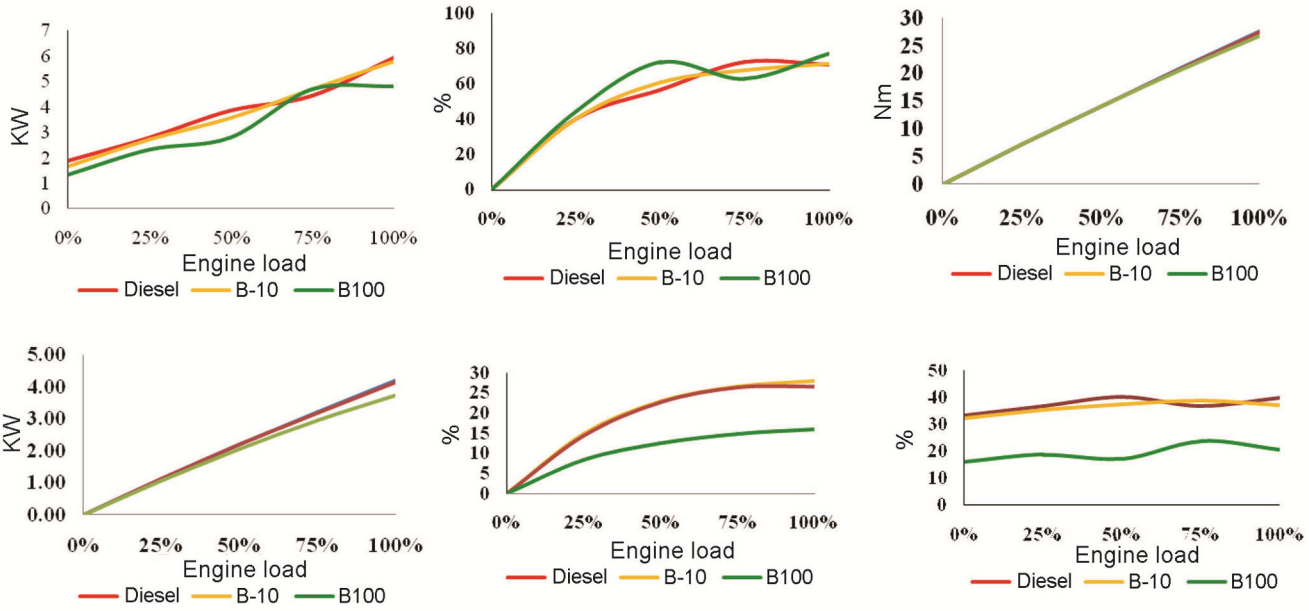

Figure 6. Performance characteristics of algal biodiesel (B100), blended biodiesel (B10) and petroleum diesel.

presence of oxygen molecule in algal biodiesel[21]. This also demonstrates the usefulness of FTIR data to segregate petroleum diesel, biodiesel and blends of biodiesel.

Fuel properties of biodiesel

A comparison was made between the fuel properties of blended (B10) biodiesel and standards for biodiesel ASTM 6751 of United States (Table 4). The fuel properties of blended biodiesel (B10) were well within the recommended limits of ASTM 6751 except distillation temperature. The cloud point which is indicator of cold flow properties was 3.0°C. The cloud point value obtained in the present study was very close (0°C) to that reported by Mostafa and El-Gendy[22] for B10 blended biodiesel produced from *Spirulina platensis*. The water content of B10 blended biodiesel was 1 ppm which was much lower than the values (43.8 ppm) reported by Mostafa and El-Gendy[22] for B10 blended biodiesel from *S. platensis*. The water content was lower than the standards (500 ppm) as well. Lower water content is a favourable attribute as higher water content causes blocking of filters and promotes microbial growth[23]. The ash content of the blended B10 biodiesel from *C. vulgaris* was 0.001% which was lower than the ash content reported for *S. platensis* (0.002%)[22]. Lower ash content is advantageous, as higher ash content deposits on engine parts and results in abrasion[24]. Kinematic viscosity of 1.45 mm^2/s was obtained for B10 blended biodiesel of *C. vulgaris* which

Table 5. Cost economics for outdoor mass culture of marine *C. vulgaris* (NIOT-74) grown in bubble column photobioreactor and raceway

Parameters	Bubble column (INR)	FRP raceway (INR)
Culture volume (l)	300.00	2000.00
Culture period (days)	10.00	10.00
Biomass (g)	900.00	3600.00
Lipid (g)	180.00	720.00
Particulars of cost elements involved in culture operation		
Seawater	100.00	666.00
Nutrient	40.00	240.00
Inoculum	4.00	24.00
Operation (electricity)	38.50	376.00
Harvesting (electroflocculation)	12.00	72.00
Drying (solar)	2.50	32.00
Labour (2 nos)	50.00	50.00
Sub total	247.00	1460.00
Asset (Raceway and BPR) depreciation @ 10% and interest on capital 10%	46.84	1095.00
Asset (Electroflocculation) depreciation @ 10% and interest on capital @ 10%	493.00	493.00
Total	786.84	3048.00
Cost of per kg dry biomass (in INR)	874.26	1693.33
Cost of per kg dry biomass (in US$)	3.72	12.40

Conversion rate 1 US$ = 70.48 INR as on 11 January 2019.

was well within the limits of the recommended standards, substantiating the suitability of the B10 blended biodiesel for fuel injection and atomization[23]. All the measured fuel properties of blended B10 diesel were within the recommended levels of standards for biodiesel ASTM D-7467 for blends.

Biodiesel-internal combustion engine test

The engine performance test was carried out for the blended biodiesel (B10), biodiesel (B100) and compared with the performance of petroleum diesel. Thermal efficiency is a crucial parameter for comparing different fuels[24]. Thermal efficiency for petroleum diesel increased with load (0% to 100%) from 32.42% to 39.77%. For B10 biodiesel it varied from 32.42% to 37.15% whereas in the case of B100 biodiesel it changed from 16.21% to 20.58% (Figure 6). Increase in thermal efficiency with increase in load is due to the increase in output power[25]. The thermal efficiency of blended (B10) biodiesel was very close to diesel. The break thermal efficiency, brake power and indicated torque also depicted similar trends (Figure 6). Therefore there was no significant difference in engine performances when microalgae biodiesel blended with petroleum diesel was used as fuel. These outcomes give strong indication that biodiesel from microalgae can be successfully used as an alternative fuel.

Test run with biodiesel

The biodiesel produced from marine *C. vulgaris* (NIOT-74) was blended (B10) with diesel and used as fuel for a vehicle. A four-wheeler (branded Qualis, Lanson Toyota) powered with B-10 blended biodiesel was tested for a distance of 200 km.

Economic analysis of outdoor cultivation

Cost of producing *C. vulgaris* biomass under outdoor conditions in different cultivation systems is listed in Table 5. The outdoor cultivation consumed lesser energy as there was no power requirement for temperature control and light intensity maintenance. Cost of 1 kg of biomass in BPR and raceway was 3.72 (INR 874.26) and 12.40 (1693.33) US$ respectively. The cost of biomass production achieved during this study was lesser than that reported for many other algal species (58.69 US$) under outdoor cultivation conditions[7,16]. The prime reason for the lower production cost can be attributed to the higher biomass obtained under outdoor conditions in these two systems and the supplementation of solar power for the paddle wheel aerator. Hence, there is greater potential for the industrial production of *C. vulgaris* (NIOT-74) under outdoor conditions.

Conclusion

The outdoor cultivation of marine *C. vulgaris* (NIOT-74) was studied in different photobioreactors and raceways. Biodiesel (B100) and blend (B10) were produced and their fuel properties were estimated and compared with standards. The engine test for the biodiesel and blend was done in two and four stoke engines. A hybrid solar powered electroflocculation system was developed for

cost-effective dewatering. A complete technological package for nutraceutical production coupled with biodiesel production from marine microalgae using the seafront facility (25 tonne raceway ponds) at Pamanji, Nellore, Andhra Pradesh, India is under development.

1. Report on the industry consumption review. Petroleum Planning and Analysis Cell, October 2018, 31–32.
2. Khan, S. A., Rashmi, Hussain, M. Z., Prasad, S. and Banerjee, U. C., Prospects of microalgae production from microalgae in India. *Renew. Sustain. Energy Rev*., 2009, **13**, 2361–2372
3. Shay, G. E., Diesel fuel from vegetable oils: status and opportunities. *Biomass Bioenergy*, 1993, **4**, 227–242.
4. Mata, T. A., Martins, A. A. and Caetano, N. S., Microalgae for biodiesel production and other applications: a review. *Renewable Sust. Energy Rev*., 2010, **14**, 217–232.
5. Tilman, D., Hill, J. and Lehman, C., Carbon-negative biofuels from low-input high-diversity grassland biomass. *Science*, 2006, **314**(5805), 1598–1600.
6. Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G. and Tredici, M. R., Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol. Bioeng*., 2009, **102**(1), 100–112.
7. Wang, S. K., Hu, Y. R., Wang, F., Stiles, A. R. and Liu, C. Z., Scale-up cultivation of *Chlorella ellipsoidea* from indoor to outdoor in bubble column bioreactors. *Bioresour. Technol*., 2014, **156**, 117–122.
8. Feng, P., Deng, Z., Fan, L. and Hu, Z., Lipid accumulation and growth characteristics of *Chlorella zofingiensis* under different nitrate and phosphate concentrations. *J. Biosci. Bioeng*., 2012, **114**(4), 405–410.
9. Sharma, A. K., Sahoo, P. K. and Singhal, S., Comparative evolution of biomass production and lipid accumulation potential of *Chlorella* species grown in a bubble column photobioreactor. *Biofuels*, 2016, **7**(4), 389–399.
10. Guillard, R. R. and Ryther, J. H., Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol*., 1962, **8**(2), 229–239.
11. Zhu, C. J. and Lee, Y. K., Determination of biomass dry weight of marine microalgae. *J. Appl. Phycol*., 1997, **9**(2), 189–194.
12. Lee, A. K., Lewis, D. M. and Ashman, P. J., Harvesting of marine microalgae by electroflocculation: the energetics, plant design, and economics. *Appl. Energy*, 2013, **108**, 45–53.
13. Folch, J., Lees, M. and Sloane Stanley, G. H., A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem*., 1957, **226**(1), 497–509.
14. Kashiwagi, T., Meyer-Rochow, V. B., Nishimura, K. and Eguchi, E., Fatty acid composition and ultrastructure of photoreceptive membranes in the crayfish *Procambarus clarkii* under conditions of thermal and photic stress. *J. Comp. Physiol. B*, 1997, **167**(1), 1–8.
15. Lam, M. K. and Lee, K. T., Potential of using organic fertilizer to cultivate *Chlorella vulgaris* for biodiesel production. *Appl. Energy*, 2012, **94**, 303–308.
16. Li, C., Yang, H., Li, Y., Cheng, L., Zhang, M., Zhang, L. and Wang, W., Novel bioconversions of municipal effluent and CO_2 into protein riched *Chlorella vulgaris* biomass. *Bioresour. Technol*., 2013, **132**, 171–177.
17. Zhou, X., Xia, L., Ge, H., Zhang, D. and Hu, C., Feasibility of biodiesel production by microalgae *Chlorella* sp. (FACHB-1748) under outdoor conditions. *Bioresour. Technol*., 2013, **138**, 131–135.
18. Dahmani, S., Zerrouki, D., Ramanna, L., Rawat, I. and Bux, F., Cultivation of *Chlorella pyrenoidosa* in outdoor open raceway pond using domestic wastewater as medium in arid desert region. *Bioresour. Technol*., 2016, **219**, 749–752.
19. Ramos, L. C., Sousa, L. J., da Silva, A. F., Falcão, V. G. O. and Cunha, S. T., Evaluation of electro-flocculation for biomass production of marine microalgae *Phaodactylum tricornutum*. *Int. J. Biol., Biomol., Agric., Food Biotechnol. Eng.*, 2017, **11**(6), 391–394.
20. Huerlimann, R., De Nys, R. and Heimann, K., Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. *Biotechnol. Bioeng*., 2010, **107**(2), 245–257.
21. Peña, A. G., Franseschi, F. A., Estrada, M. C., Ramos, V. M., Zarracino, R. G., Loría, J. C. Z. and Quiroz, A. V. C., Fourier transform infrared-attenuated total reflectance (FTIR-ATR) spectroscopy and chemometric techniques for the determination of adulteration in petrodiesel/biodiesel blends. *Química Nova*, 2014, **37**(3), 392–397.
22. Mostafa, S. S. and El-Gendy, N. S., Evaluation of fuel properties for microalgae *Spirulina platensis* bio-diesel and its blends with Egyptian petro-diesel. *Arabian J. Chem*., 2017, **10**, S2040–S2050.
23. Knothe, G. and Steidley, K. R., Kinematic viscosity of biodiesel fuel components and related compounds. Influence of compound structure and comparison to petrodiesel fuel components. *Fuel*, 2005, **84**(9), 1059–1065.
24. Lapuerta, M., Armas, O. and Rodriguez-Fernandez, J., Effect of biodiesel fuels on diesel engine emissions. *Prog. Energy Combust. Sci*., 2008, **34**(2), 198–223.
25. Yasar, F. and Altun, S., The effect of microalgae biodiesel on combustion, performance, and emission characteristics of a diesel power generator. *Thermal Sci.*, 2018, **22**(3), 1481–1492.

ACKNOWLEDGEMENT. We thank the Ministry of Earth Sciences, Government of India, for financial support.

doi: 10.18520/cs/v118/i11/1731-1738