Bioresource Technology 216 (2016) 793–800

Contents lists available at ScienceDirect

Bioresource Technology

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

Exploration of upstream and downstream process for microwave assisted sustainable biodiesel production from microalgae *Chlorella vulgaris*

Amit Kumar Sharma [a,*], Pradeepta Kumar Sahoo [b], Shailey Singhal [c], Girdhar Joshi [d]

[a] Biofuel Research Laboratory, University of Petroleum and Energy Studies, Bidholi, Dehradun 248007, India
[b] Department of Farm Machinery & Power, Orissa University of Agriculture & Technology (OUAT), Bhubaneswar 751003, India
[c] Department of Chemistry, University of Petroleum and Energy Studies, Bidholi, Dehradun 248007, India
[d] Research and Development Department, University of Petroleum and Energy Studies, Bidholi, Dehradun 248007, India

HIGHLIGHTS

- Upstream and downstream process in *Chlorella vulgaris* has been explored for microwave assisted sustainable biodiesel production.
- 92.53% harvesting efficiency was achieved by a combined method of flocculation and filtration.
- 84.03% yield obtained by acid-based catalyzed transesterification in microwave assisted reactor.
- Microwave assisted transesterification was more efficient than conventional heating transesterification.
- *Chlorella vulgaris* can produce 13.62 ton/hectare/year biodiesel.

ARTICLE INFO

Article history:
Received 31 March 2016
Received in revised form 2 June 2016
Accepted 3 June 2016
Available online 5 June 2016

Keywords:
Chlorella vulgaris
Open raceway cultivation
Microwave irradiation
Biodiesel

ABSTRACT

The present study explores the integrated approach for the sustainable production of biodiesel from *Chlorella vulgaris* microalgae. The microalgae were cultivated in 10 m^2 open raceway pond at semi-continuous mode with optimum volumetric and areal production of 28.105 kg/L/y and 71.51 t/h/y, respectively. Alum was used as flocculent for harvesting the microalgae and optimized at different pH. Lipid was extracted using chloroform: methanol (2:1) and having 12.39% of FFA. Effect of various reaction conditions such as effect of catalyst, methanol:lipid ratio, reaction temperature and time on biodiesel yields were studied under microwave irradiation; and 84.01% of biodiesel yield was obtained under optimized reaction conditions. A comparison was also made between the biodiesel productions under conventional heating and microwave irradiation. The synthesized biodiesel was characterized by ^{1}H NMR, ^{13}C NMR, FTIR and GC; however, fuel properties of biodiesel were also studied using specified test methods as per ASTM and EN standards.

1. Introduction

Energy security, environmental concerns, increase in industrialization and living standards of the society across the world are the major reasons to be considered as driving force to look for the alternative energy sources. Fossil fuels are the only primary source of energy and have been continuously used globally for the last few decades. The exhaustive exploitation and consumption of fossil fuels has resulted in discharge of these resources (Karpagam et al., 2015). Thus alternative renewable energy sources like wind, solar, hydropower, geothermal, hydrogen, nuclear and biomass have attracted a remarkable attention globally, among these renewable energy sources, biomass (including both the agriculture and forestry) based materials have been studied extensively to produce the liquid fuels as an alternate of petroleum based fuels (Anandarajah et al., 2012; Kang et al., 2015). Biodiesel is considered as one of the most promising liquid biofuel produced either from edible/non-edible oil seed or from algae, among them microalgae based biofuels have been emerging as promising alternative energy sources due to higher photosynthetic rate, ability to grow in saline or waste water, higher growth rate, and lipid accumulation in the form of triacylglycerides (TAG) (Anandarajah et al., 2012, Chisti, 2007, Kang et al., 2015). Furthermore, the lipid extracted from microalgae is similar to straight vegetable oil in chemical

* Corresponding author.
E-mail address: amit.orgchemistry@gmail.com (A.K. Sharma).

http://dx.doi.org/10.1016/j.biortech.2016.06.013
0960-8524/

composition and have a great potential to replace first and second generation feedstock (e.g. Soyabean, Rapeseed, *Jatropha curcus* etc.) for biodiesel production. Microalgae can be grown anywhere with sufficient sunlight (Becker, 1994; Nigam and Singh, 2010) and do not compete with food crops, agricultural land and water. Additionally, Microalgae have several folds higher biomass productivity compared to terrestrial energy crops (Demirbas, 2009). Microalgae have a biomass productivity of ~50–80 Mt/ha/year in open raceway ponds and productivity ~150 Mt/ha/year in photobioreactors; however, the biomass productivity of other energy crops such as soybeans, *Calophyllum inophyllum*, Jatropha is estimated as 3–4 Mt/ha/year, 3–10 Mt/ha/year, and 0.1–10 Mt/ha/year, respectively (Adesanya et al., 2014; Khudhair and Farid, 2011; Rashid et al., 2014). In addition, microalgae have a very short harvesting period (1–10 days depending upon algae species) in compression to conventional crop plants which are usually harvested once or twice a year (Chisti, 2007; Khan et al., 2009). Moreover, microalgae have more photosynthetic efficiency than terrestrial plants and 1 kg of microalgae biomass can fix 1.8 kg of carbon dioxides (Khan et al., 2009). These are the reasons which make algal biofuels technology more attractive than energy crops based biofuels.

One of the most important key factors in microalgae based biofuel production is the cultivation technology of microalgae. Microalgae are generally cultivated in both open ponds and closed photobioreactor. However, closed photobioreactor is more efficient in terms of biomass productivity, but the capital cost and operational expanses of algae cultivation in photobioreactor is much higher than open ponds (Adesanya et al., 2014). Therefore, open ponds are considered the most attractive route for large scale biomass production as it is easy to construct and operate (Kumar et al., 2015). Since due to microscopic size of microalgae cells (1–10 μm diameters) the density of these microalgae cells is almost equal to the density of water in dilute culture medium, hence its harvesting is another challenging area for algae based biodiesel and it contributes approximately 20–30% of total biodiesel production cost (Kim et al., 2013). Various harvesting techniques like centrifugation, coagulation, flocculation, flotation, filtration, electro-flotation, electrophoresis, and ultrasound have been extensively studied and proven in the past for the economical algal biodiesel production. Lipids extracted from microalgae have higher free fatty acids (FFA) and therefore, two step esterification and transesterification process is generally used for biodiesel production (Veeramuthu et al., 2014a,b; Suganya et al., 2013). Biodiesel is generally produced by base catalyzed transesterification of triglycerides with alcohol under conventional heating; however, the higher energy consumption and longer reaction time are the major drawback of conventional heating assisted biodiesel production. The heat transfer in conventional heating is inefficient and it occurs through convection, conduction and radiation from the surfaces of the reactor. To overcome these issues, microwave assisted transesterification process are emerging as the most energy efficient, quick and reliable process for biodiesel production (Patil et al., 2013). Heat transfer in microwave assisted process usually takes place by dipolar polarization, ionic conduction and interfacial polarization mechanisms to enhance the localized and rapid heating of reaction materials (Kumar et al., 2015).

The present study focused on the integrated approach to study the upstream and downstream processes of biodiesel production from *Chlorella vulgaris* microalgae. Mass cultivation of microalgae is successfully done in open raceway ponds at semi-continuous mode. Harvesting of microalgae is developed using alum as flocculent followed by filtration. Biodiesel production process is optimized under microwave irradiation and compared with conventional heating. Characterization of synthesized biodiesel is carried out using GC, FTIR, ^{1}H NMR, and ^{13}C NMR. Fuel properties of microalgae biodiesel are examined by using standard test methods as prescribed by ASTM and BIS EN standards.

2. Materials and methodology

2.1. Microalgae strain and pre-cultivation conditions

Pure cultures of *Chlorella vulgaris* was obtained from Vivekananda Institute of Algal Technology (VIAT), Chennai (India). The stock culture of microalgae strains was maintained regularly on agar slants and liquid medium using sterilized BBM (Bold's Basal Medium) medium (with initial pH of 6.8) under laboratory conditions [*i.e.* 24 °C (±1 °C) under (~2500 l×) light intensity and 16/8 light dark cycle in a photobioreactor].

2.2. Mass cultivation of Chlorella vulgaris in open raceway ponds at semi-continues mode

Cultivation of microalgae *Chlorella vulgaris* was carried out in a concrete raceway pond of dimension 5 * 2 * 0.5 m^3 ($l * b * h$) with a total working volume of 1200 L. Initially the seed culture of *Chlorella vulgaris* was prepared in the flat plate photo (FPP) bioreactor photobioreactor using BG-11 as growth media; however, BG-11 was found more expensive at large scale hence more economical fertilizer based nutrient media, having composition urea 250 mg/L, DAP (Di ammonium phosphate) 250 mg/L, potash (potassium Chloride) 250 mg/L, magnesium sulphate 250 mg/L, sodium carbonate 20 mg/L, Ferric citrate 6 mg/L and micronutrient from BG-11 medium (half strength), was prepared and used for cultivation. Initially the pH of the culture was maintained at pH 7. Fresh water was used for the cultivation of algae and was thoroughly sterilized and de-chlorinated by treating with sodium hypochlorite and sodium thiosulphate solutions. Cultivation of microalgae algae was done in semi continuous mode and started with 0.300 g/L of initial concentration, and the culture was agitated with a paddle wheel having a speed of 15 rpm to proper dissolution of CO_2 and also to avoid the settling of algal strain. During cultivation process the algae was initially grown for 12 days at batch mode and 50% of microalgae culture was replaced subsequently by freshly prepared nutrient media at every 6th day. This experiment was conducted for a period of 48 days. Total six harvestings were obtained during semi-continuous mode. After each harvesting, pH was adjusted 7–8. Contaminations were regularly checked with a microscope.

2.3. Harvesting

The microalgae cells were harvested via two-step process *i.e.* flocculation and filtration. To optimize the suitable dose of flocculation, preliminary study was done in 100 ml test tube using Potassium alum [$(KAl(SO_4)_2{\cdot}12H_2O$] as flocculent.

Effect of concentrations of potassium alum on its flocculation efficiency with respect to different time intervals (15, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min) were carried out with different concentration of potassium alum *i.e.* 50, 100, 150, 200, 250, 300, 400 and 500 mg/L respectively. Besides, effect of pH on flocculation efficiency was also studied. Prior to use as large scale (5 L flask, 100 L and 200 L plastic vessel) the optimized conditions were first tested for 100 mL reaction mass. Concentrated microalgae slurry obtained after flocculation was filtered through filter cloth and washed with fresh ground water 3–4 times to remove excess salts in microalgae biomass.

The flocculating efficiency was determined using following Eq. (1):

$$\text{Flocculating efficiency}\,(\%) = \left(1 - \frac{OD_b}{OD_a}\right) \times 100 \qquad (1)$$

where, OD_b and OD_a are the optical density of the algal culture before and after the flocculation analyzed at 680 nm.

2.4. Lipid extraction

Lipid extraction was carried out in soxhlet apparatus using following optimisation condition: chloroform: methanol (2:1) as solvent, 10:1 solvent to biomass ratio and 8 h time duration (data not shown here for optimization). Microalgae cell membrane acts as a barrier and reduces the recovery of total lipid extraction; therefore, microwave cell disruption processes (pre-treatment methods) were applied before extraction in order to achieve high lipid efficiency. In this method, the microalgae biomass was treated in microwave reactor for 15 min at 100 °C. Microwave pre-treated algae biomass was employed to soxhelt extraction unit. Under these optimized conditions, an average lipid yield of 22.68% of dry biomass was obtained. The saponification value of extracted microalgae lipid was 194.87 mg KOH/g while free fatty acid (FFA) and acid value of lipid was observed up to 12.39% and 24.67 mg KOH/g respectively.

2.5. Process optimization of biodiesel production from microalgae biomass

Transesterification of Microalgae lipid was carried out in a microwave (MW) reactor manufactured by Ragatech. Microwave reactor was equipped with a three neck flask with reflux condenser, a magnetic stirrer and a non-contact infrared temperature control system. Each experiment was repeated three times to evaluate the reproducibility of microwave effect. After each experiment, microwave reactor was allowed to cool and returned to original conditions. Crude microalgae lipid, extracted by soxhlet apparatus, was re-dissolved in n-hexane, filtered with Whatman-40 and concentrated in vacuum evaporator. Since, microalgae lipid has higher acid value, biodiesel production was carried out in two steps. First step of biodiesel production process was esterification for reduction of FFA below 1% and second step is the transesterification of lipid for biodiesel production using base catalyst.

2.5.1. Acid-catalyzed esterification (conversion of FFA to triglyceride)

Acid-catalyzed esterification was carried out at 60 °C and atmospheric pressure in microwave reactor with constant agitation using magnetic stirrer. Optimization study of acid catalyzed esterification was carried out with different concentrations of acid catalyst (0–1.5 vol.%), lipid to methanol ratio (1:10, v/v) and reaction time intervals (5–40 min). Mixture was stirred with constant speed and refluxed continuously for proper mixing and to decrease chances of solvent vapor loss during reaction. Reaction progress was frequently examined by analyzing acid value content at different at pre-determined time intervals. Once FFA (free fatty acid content) was decreased below 2%, reaction was stopped and end products of the reaction were poured into the separating funnel. Lower layer was thrown out while upper layer is concentrated in vacuum evaporator. Approximately, 1 ml sample was taken from this concentrated solution and washed with hot water to eliminate the impurities, re-extracted with hexane for Gas Chromatograph (GC) analysis.

2.5.2. Base-catalyzed transesterification

Transesterification of the product of acid esterification stage was conducted under microwave heating using an alkali catalyst (KOH) at 700 W power. To optimize and improving biodiesel yield, various experiments were performed under following reaction conditions: catalyst concentration of 0.5–2.5 (wt/vol.%), lipid to methanol molar ratio (1:4–1:14 v/v), temperatures (45–65 °C) and reaction time of 5–25 min. After completion of reaction, biodiesel sample was collected in a separating funnel and washed three times with lukewarm distilled water having 1% acid (HCl) to remove glycerol, the unreacted catalyst and other impurities. After washing, moisture content of biodiesel was removed using sodium sulphate. Biodiesel sample was centrifuged at 6000 rpm for 10 min to remove impurities and sodium sulphate, filtered through Whatman-40 filter paper and collected in a sealed glass bottle. All the experiments were conducted three times, and average value with ± standard deviations was represented as results in graphs. Biodiesel yield was examined gravimetrically as Eq. (2).

$$\text{Biodiesel yield}\,(\text{wt}\%) = \frac{\text{wt of biodiesel}\,(\text{g})}{\text{wt of oil}\,(\text{g})} \times 100 \quad (2)$$

2.6. Analysis of energy consumption in microwave assisted biodiesel reactor and conventional heating assisted reactor

Energy consumption in microwave assisted reactor was calculated by multiplying watt with time while in conventional reactor, energy consumption pattern for bio-diesel production has been recorded with the help of Power Analyser (ALM-35).

2.7. Analysis of biodiesel composition using gas chromatograph

Gas chromatograph (Nucon 5700 series) equipped with Flame ionization detector (FID) using EOX column (serial no 5061; 30 m length, 0.25 mm ID and 0.25 mm outer dia) was used for analysis of biodiesel composition. Pure nitrogen (99.999%) was used as carrier gas with a flow rate of 30 mL/min. The oven temperature was set at 160 °C for 2 min, followed by 4 °C/min ramp up to 240 °C and maintained for 40 min. The injector and FID detector temperature was set at 240 °C and 220 °C respectively. Supelco 37component FAME mix (Sigma–Aldrich, USA) was used as standard.

2.8. FTIR and NMR characterisation of biodiesel

Chlorella vulgaris biodiesel was characterized by thermo Nicolet FT-IR spectrophotometer in the range of υ_{max} = 4000–400 cm^{-1}. NMR analyses were made by BRUKER 400.13 MHz spectrometer equipped using CDCl3 and TMS as solvent and internal standard respectively. ^1H (300 MHz) spectra were recorded with pulse duration of 30 s, a recycle delay of 1.0 s and 6 scans. The ^{13}C (100.47 MHz) spectra were recorded with a pulse duration of 30, a recycle delay of 1.89 s and 256 scans.

2.9. Analysis of fuel properties of microalgae biodiesel

Fuel properties of biodiesel such as kinematic viscosity, density, flash point, pour point, moisture content, calorific value, copper strip corrosion and oxidation stability were characterized using standard protocols and compared with ASTM D6751 and IS: 15607. All tests were run in duplicate and mean values are reported.

3. Result and discussion

This investigation explored the cultivation of *Chlorella vulgaris* in open raceway ponds, optimisation of harvesting method, optimisation of biodiesel production in microwave assisted reactor and analysis of fuel properties of microalgae biodiesel to explore upstream and downstream process in *Chlorella vulgaris* for biodiesel production.

3.1. Microalgae cultivation in open raceway ponds at semi-continuous mode

Large scale cultivation of microalgae was carried out in 1200 L capacity open raceway ponds at semi-continuous mode. Tape water sterilized with the help of sodium hypochlorite and dechlorinated by sodium thiosulphate was used for preparation of commercial grade fertilizer based nutrient media. During semi-continuous cultivation, the microalgae was initially grown for 12 days at batch mode and then 50% of microalgae culture was replaced by the same volume of nutrition rich growth media at every six days interval. Total six harvestings were carried out during semi-continuous cultivation. After each harvest pH was adjusted from 7.0 to 8.0. During the experiment, average Temperature was 20–34 °C. As shown in Fig. 1, optimum volumetric and areal productivity achieved by *Chlorella vulgaris* in the semi-continuous mode was 77 mg/L/d and 19.61 g/m^2/d respectively. In addition, lipid productivity was 11.52 mg/L/d that was 30.60% higher than batch mode. Moreover, the specific growth rate and division per day were observed 0.1111 and 0.160 respectively for *Chlorella vulgaris*. Veeramuthu et al., 2014a,b cultured *Microcystis aeruginosa* in 5000 L capacity raceway ponds at semi-continuous mode and observed biomass productivity up to 28 g/m^2/day, which is higher than biomass productivity (19.61 g/m^2/d) achieved in this study. Bhowmick et al., 2014 culture *Chlorella variabilis* in open raceway ponds with average biomass productivity of 5.78 g/m^2/day. Veeramuthu et al. (2015) achieved biomass productivity of alga *Botryococcus sudeticus* 23.7 g/m^2/d within 12 days at batch mode. It is well reported that biomass productivity changes species to species and is highly affected by temperature fluctuation within a diurnal cycle and seasonally temperature variation under outdoor cultivation. In Dehradun, there is more variation in temperature between night and days. This may be the possible reason for lower biomass productivity of *Chlorella vulgaris*. However, biomass productivity of *Chlorella vulgaris* can be increased further by increasing and proper mixing management of carbon dioxide supplementation.

3.2. Optimisation of harvesting process

Microalgae biomass was harvested via flocculation and filtration, followed by drying under sunlight. Experiments were performed with different concentrations of alum (50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L, 250 mg/L, 300 mg/L, 400 mg/L and 500 mg/L) with varying the time (15 min, 30 min, 60 min, 90 min, 120 min, 150 min, 180 min, 210 min, 240 min, 270 min and 300 min). However, maximum biomass was harvested when 500 mg/L alum was used as flocculent (82.64%), but 250 mg/l alum concentration was considered as optimized concentration because it also gave approximately same results (82.10%) (Fig. 2). Furthermore, excess of alum concentration resulted in higher harvesting cost.

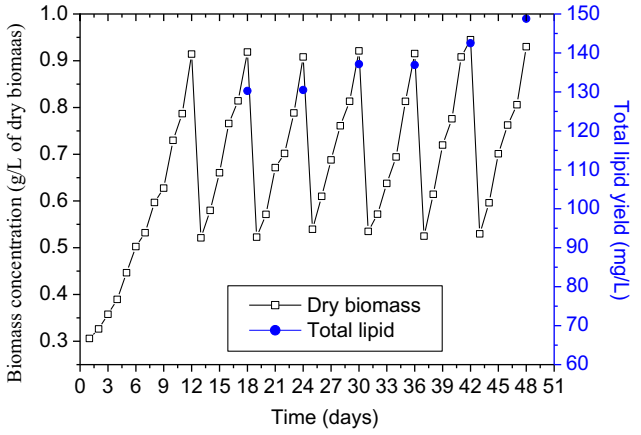

Fig. 1. Mass cultivation of *Chlorella vulgaris* in open raceway ponds at semi-continues mode.

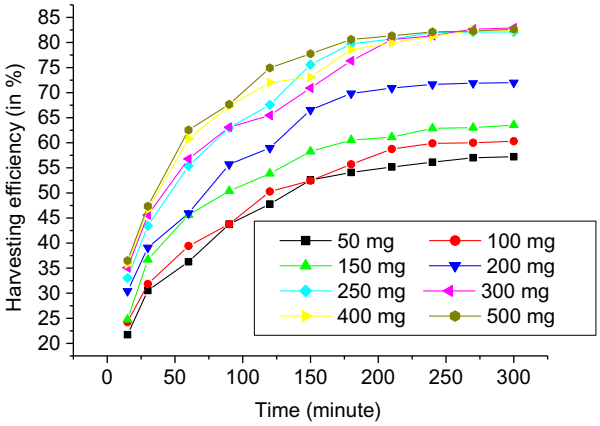

Fig. 2. Effect of alum concentration on biomass harvesting with time.

To increase flocculation efficiency, effect of pH on harvesting efficiency was also studied at room temperature. It was observed that alum gives best results in acidic medium and 92.53% flocculation efficiency was achieved at pH 5 by a combined method of flocculation and filtration. After flocculation, the dense culture medium was filtered through filter cloth having 2–10 μm pores. When these conditions were applied to large scale (5 L, 100 L and 200 L capacity drum), harvesting efficiency was found to be 91.63%, 89.24% and 88.78% respectively. Similarly, Bhowmick et al. (2014) found that *Chlorella variabilis* gives best flocculation results with 160 mg/L alum at pH 4. Here, the cost of microalgae harvesting was found 0.70 US$/kg which has been reported as much lower than plate centrifuge (0.80 US$/kg) and other flocculants (Granados et al., 2012). However, it needs further research to reduce the separation cost and improve harvesting efficiency for making algal biofuel technology more viable at industrial scale.

3.3. Process optimization of biodiesel production from Chlorella vulgaris lipid

Due to higher FFA content of algal lipid (12.39%), a two-step process *i.e.* acid-catalyzed esterification followed by base-catalyzed transesterification was employed for biodiesel production.

3.3.1. Acid catalyzed esterification

The acid value of *Chlorella vulgaris oil* was 24.67 mg KOH/g. The higher acid value leads to soap formation, loss of biodiesel yield and difficulties in product separation during transesterification reaction. Therefore, acid esterification pretreatment was employed to reduce acid value less than 1 mg KOH/g. Optimization study was conducted in microwave assisted biodiesel reactor at atmospheric pressure using methanol to lipid ratio 1:10, 1.5% H_2SO_4 (v/v) as acid catalyst at 60 °C for reaction duration of 5–40 min. At different time intervals, sample was taken out from reactor to analyze FFA content. Once FFA level was reached to target value (less than 1%), the reaction was stopped. The results of the reaction were summarized in Fig. 3. It was observed that FFA level of microalgae lipid was decreased up to 0.93% with 24.4% production yield of biodiesel within 40 min. The results was supported by the study of Jaliliannosrat et al., 2013 who reduced FFA of Jatropha oil from

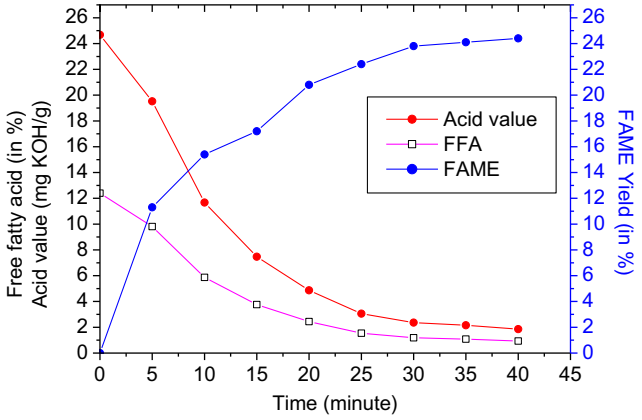

Fig. 3. Effect of acid catalyst on FFA reduction and biodiesel yield.

14% to less than 1% using H_2SO_4 as acid catalyst after 35 minute in microwave assisted reactor (Jaliliannosrat et al., 2013) Similarly, Suganya et al. (2013) worked on microalgae *Enteromorpha compressa* and achieved FFA reduction from 6.3% to 0.34% using following optimized reaction conditions: 1.5% H_2SO_4, 12:1 methanol to lipid ratio, 60 °C temperature and 1.5 h of reaction time. Tiwari et al. (2007) investigated that 1.43% v/v H_2SO_4 acid catalyst, 0.28 v/v methanol-to-oil ratios and 88-min reaction time at 60 °C was the optimum condition to reduce the high FFA level of Jatropha oil from 14% to less than 1%. Veeramuthu et al. (2015) reduced microalgae lipid FFA level from 11.5% to 0.6% using 1.5% of sulfuric acid and 150 min reaction time.

3.3.2. Base catalyzed transesterification

The above esterified algal lipid was further preceded for biodiesel (FAME) production via base catalyzed transesterification. Reaction parameters such as catalyst amount, molar ratio of lipid to methanol, reaction temperature and time were analyzed in a microwave assisted reactor to get maximum biodiesel yield from *Chlorella vulgaris* lipid.

To analyze the effect of catalyst on FAME yield, a base catalyst, potassium hydroxide was used in the range of 0.5–2.5% in the present experimental analysis. Other initial parameters were kept as follows: methanol to algal lipid molar ratio 8:1, reaction temperature 55 °C and reaction time 20 min. Effect of catalyst amount on FAME yield is shown in Table 1. FAME yield was increased dramatically from 41.15% to 63.03% with increase of catalyst concentration from 0.5% to 1.5%. Further increase in catalyst concentration resulted in lower fame yield and it was found that FAME yield was reduced up to 47.49% at catalyst concentration of 2.5% (Table 1). This can be explained by the fact that addition of excess KOH catalyst gave rise to the formation of an emulsion that increased the solution viscosity and in turn generated a gel. Similar, results obtained by chen et al. who observed maximum biodiesel yield from *Scenedesmus* lipid using 2% potassium hydroxide as base catalyst (Chen et al., 2012). Suganya et al. found that increasing concentration of NaOH more than 1% resulted in decrease of biodiesel yield. It was also observed that insufficient amount of catalyst also gave rise to lower FAME yield. Therefore, optimum concentration of alkali catalyst was observed 1.5% in this study.

The amount of alcohol added to algal lipid is one of the important factors that affects FAME (biodiesel) yield as well as production cost of biodiesel. The effect of methanol to algal lipid ratios (4:1–14:1v/v) on biodiesel yield was examined here. Other initial conditions were kept constant *i.e.* catalyst concentration 1.5%, time 20 min and reaction temperature 55 °C. As shown in Table 1, FAME yield is seen increase from 50.06% to 71.79% as methanol to lipid ratio was increased from 4:1 to 10:1. This may be due to due to the fact the methanol evaporates just above this value, thus rendering unavailable for reactions in the liquid media. No significance change was observed in FAME yield beyond 10:1 M ratio of lipid methanol. Moreover, high volume of methanol would also have an impact on biodiesel production cost and interfere with the purification of biodiesel. Hence, methanol to lipid molar ratio 10:1 was found to be the best condition to get maximum FAME yield of 71.79%. These results are agreed by Silitonga et al. who found optimum methanol to oil ratio 12:1 to achieve efficient yield (Silitonga et al., 2013).

Table 1
Parameter affecting microwave assisted transesterification: effect of catalyst concentration, temperature, solvent to lipid ratio and time duration on biodiesel yield.

S. No.	Catalyst amount (%wt)	Reaction temperature (°C)	Methanol/Oil ratio	Reaction time (min)	Biodiesel yields (%)
1	0.5	55	8:1	20	41.15
2	1	55	8:1	20	60.52
3	1.5	55	8:1	20	63.03
4	2	55	8:1	20	57.48
5	2.5	55	8:1	20	47.49
6	1.5	55	04:01	20	50.06
7	1.5	55	06:01	20	60.96
8	1.5	55	08:01	20	65.62
9	1.5	55	10:01	20	71.79
10	1.5	55	12:01	20	72.05
11	1.5	55	14:01	20	72.56
12	1.5	45	10:01	20	52.10
13	1.5	50	10:01	20	64.60
14	1.5	55	10:01	20	72.44
15	1.5	60	10:01	20	84.08
16	1.5	65	10:01	20	84.13
17	1.5	60	10:01	2.5	41.57
18	1.5	60	10:01	5	56.02
19	1.5	60	10:01	7.5	64.47
20	1.5	60	10:01	10	78.12
21	1.5	60	10:01	12.5	81.28
22	1.5	60	10:01	15	84.01
23	1.5	60	10:01	17.5	84.83
24	1.5	60	10:01	20	85.58
25	1.5	60	10:01	22.5	83.83
26	1.5	60	10:01	25	85.57

In order to study the effect of reaction temperature, transesterification was carried out at different temperature (45–65 °C) for 20 min. Other parameters such as methanol to lipid ratio 10:1 and KOH concentration 1.5% (w/v) were used. The results revealed that FAME yield increased with rise in temperature (Table 1). When temperature was increased from 45 °C to 60 °C, FAME yield raised from 52.1% to 84.08%. Further increase in temperature does not have significant effect on FAME yield and makes it energy expensive. Therefore, 60 °C was found to be the optimum temperature condition. This may be due to the fact the methanol evaporates just above this value, thus rendering unavailable for reactions in the liquid media. Leung and Guo (2006) studied the impact of different temperature conditions (30–70 °C) on the FAME yield. They found that 60 °C was the optimum temperature with maximum biodiesel yield of 88.8% (Leung and Guo, 2006).

To increase the efficacy of the reaction, transesterification was also carried out with different time durations (2.5–25 min). In this part, experiments were conducted with catalyst concentration (1.5%), methanol to lipid molar ratio (10:1), temperature (60 °C) and varied reaction time of 3–30 min. Results are shown in Table 1. It was observed that with increasing reaction time from 2.5 min to 15 min, FAME yield was increased from 41.57% to 84.01%. Beyond 15 min, even after 17.5 min there was no obvious change in FAME yield. Hence, time duration of 15 min is sufficient to achieve efficient yield. Similarly, Kanitkar et al. (2011) achieved 98.82% yield within 20 min from rice barn oil using NaOH as catalyst at 80 °C reaction temperature. Teo and Idris, 2014 obtained maximum biodiesel yield of 83.33% and 77.14% for *Nannochloropsis* sp. and *Tetraselmis* sp. using simultaneous cooling and microwave heating (SCMH) method using 50 °C, 800 W, 16 h time duration and 1:12 lipid to solvent ratio.

After completion of reaction, the end product of reaction were poured in separating funnel for 12 h to separate glycerol, excess of methanol and other impurities. This time upper layer holds biodiesel and lower layer glycerol and other impurities. After draining lower layer, upper biodiesel layer was separated carefully and washed three times with slightly hot distilled water containing 1% acid. To remove moisture of biodiesel, it was treated with anhydrous sodium sulphate. For that, mixture of biodiesel and sodium sulphate was centrifuged at 8000 rpm for 10 min and then, filtered through Whatman filter paper to get purified and clean biodiesel. Characterisation of the synthesized biodiesel was carried out using GC, NMR (^{13}C and ^{1}H) and FTIR techniques.

To compare conventional and microwave assisted process, biodiesel was also prepared in a conventional reactor, commonly used for biodiesel synthesis, under same optimized condition as stated in above section. Variation in reaction time was the only parameter studied and the reaction was carried out for 3 h to get maximum yield. Energy consumption for biodiesel production (esterification and transesterification) was found to be 0.641 KWh for microwave reactor which is lesser than conventional heating reactor (Table 2). Furthermore, reaction time of microwave assisted transesterification was much lower than that of conventional biodiesel synthesis causing the production rate of biodiesel of microwave synthesis at least 12 times higher than that of conventional synthesis. This can be explained by the fact that microwaves transfer energy into reactants (oil and methanol) by dipolar polarization, ionic conduction, and interfacial polarization mechanism, resulting in large reduction of activation energy due to better molecular level interaction of the microwaves in the reaction mixture (Gude et al., 2013; Mazzocchia et al., 2004). Many researchers also support the fact that microwave assisted transesterification takes place at faster rate with energy saving as compared to the conventional oil/water bath heating assisted transesterification for biodiesel production (Gude et al., 2013; Mazzocchia et al., 2004; Patil et al., 2013).

Table 2
Comparative analysis of microwave assisted transesterification and conventional transesterification.

S. No.	Characteristic/ parameter	Microwave reactor	Conventional reactor (oil bath heating reactor)
1	Reaction duration	15 min	3 h
2	Biodiesel yield	84.01%	83.23%
3	Energy consumption (esterification and transesterification)	0.641 KWh	0.684 KWh
4	Advantages	Short reaction time, reduce product separation time, cleaner products, and energy efficient	Simple operation, use of low energy source

3.4. Characterization of microalgae biodiesel

3.4.1. GC analysis of microalgae biodiesel

Fatty acid composition of *Chlorella vulgaris* algal biodiesel is shown in Table 3. It was observed that *Chlorella vulgaris* biodiesel had 33.53% saturated fatty acid, 28.68% monounsaturated fatty acid and 37.12% polyunsaturated fatty acid composition. GC analysis showed that microalgal biodiesel had following fatty acids: caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, and erucic acid. According to European standards EN14214, an ideal biodiesel should not have more than 12% and 1% linolenic acid (C18:3) and polyunsaturated FA (>4 double bond) respectively. In the present study, microalgae biodiesel had 7.54% linolenic acid which under limit of European standards EN14214. Furthermore, presence of higher saturation content of FAME in this strain makes it more suitable for biodiesel production.

3.4.2. ^{1}H nuclear magnetic resonance analysis

NMR spectroscopy was used to explain the structure and chemical properties of biodiesel (S1). A strong singlet signal at $\delta = 3.66$ ppm indicates the protons of the methyl group of *Chlorella vulgaris* biodiesel. This peak was absent in the lipid spectrum. Another multiplet resonance was observed at $\delta = 0.86–0.88$ ppm which represents terminal methyl protons ($C–CH_3$). Multiplet resonance detected at $\delta = 5.3$ ppm signifies the proton attached to the olefinic carbon (one double bond). Multiplet at $\delta = 1.61$ ppm indicates β-methylene protons to ester bond ($CH_2–C–CO_2Me$) and a

Table 3
Fatty acid methyl ester composition of microalgae biodiesel.

S. No.	Fatty acid methyl ester	No. of carbon atoms	Composition of microalgae (*Chlorella vulgaris*) biodiesel (in%)
1	Caprylic acid methyl ester	C-8.0	0.09
2	Capric acid methyl ester	C-10.0	0.73
3	Lauric acid methyl ester	C-12.0	0.19
4	Myristic acid methyl ester	C-14.0	2.04
5	Palmitic acid methyl ester	C-16.0	24.31
6	Palmitoleic acid methyl ester	C-16:1	0.63
7	Stearic acid methyl ester	C-18.0	5.90
8	Oleic acid methyl ester	C-18.1	24.52
9	Linoleic acid methyl ester	C-18.2	29.57
10	Linolenic acid methyl ester	C-18.3	7.54
11	Arachidic acid methyl ester	C-20	0.25
12	Behenic acid methyl ester	C-22	nd
13	Erucic acid methyl ester	c-22:1	3.52
14	Saturated fatty acid methyl esters	–	33.53
15	Mono unsaturated fatty acid methyl ester	–	28.68
16	Poly unsaturated fatty acid methyl esters	–	37.12

strong resonance at δ = 1.30 ppm was due to the protons of backbone methylene protons of aliphatic fatty acid chain. Obviously, absence the peaks for glyceride protons at δ = 4.2–4.3 ppm and the presence of methyl resonance at δ = 3.66 ppm confirm the conversion of lipid into biodiesel. The obtained analytical results are in support of O'Donnell et al. who had done the similar analytical work with palm oil and soybean oil biodiesel.

3.4.3. ^{13}C nuclear magnetic resonance analysis

The ^{13}C NMR spectrum of *Chlorella vulgaris* biodiesel (S1) showed that a characteristic peaks at δ = 174.3 ppm represents the ester carbonyl carbon (–COO–). Another peak at δ = 51.4 ppm indicates methoxy carbon (–OMe) groups of *Chlorella vulgaris* biodiesel. Cluster of signals at δ = 127.8, 128.0, 128.1, 128.7, 129.6, 129.9 and 130.1 ppm designates the olefinic carbons which confirm the unsaturation present in biodiesel. Some peaks were also observed in the range from δ = 13–34 ppm which indicates methyl (terminal) and methylene (backbone of biodiesel) carbons of fatty acid moiety.

3.4.4. FTIR (Fourier transform infrared spectroscopy) analysis

FTIR was used to ascertain the functional groups and their shift in the lipid and biodiesel of *Chlorella vulgaris* (S2). Peaks observed at ν_{max} = 2857.11 and 2924.77 cm^{-1} represent the symmetrical and asymmetrical –CH_2– stretching and thus confirm the existence of lipid (–CH_2– groups form the backbone in lipids). A strong signal at ν_{max} = 1741.22 cm^{-1} indicates the stretching of carbonyl group (–C═O) in biodiesel. A weak signal at 3005.07 cm^{-1} was due to the olefinic group (═CH–) and indicates the presence of unsaturated fatty acids in methyl ester. Peaks observed at ν_{max} = 1247.75 cm^{-1} and 1176.71 cm^{-1} represents C–O stretching and absorption at ν_{max} = 1368.16 cm^{-1} indicates the methyl group (–CH_3–). A sharp absorption peak at ν_{max} = 723.46 cm^{-1} is suggestive of –CH_2-rocking.

3.5. Analysis of physico-chemical properties of microalgae biodiesel

To run biodiesel successfully in compression–ignition (CI) diesel engines, its physico- chemical properties should meet the international standard specifications (e.g. ASTM D6751, EN 14214 and IS: 15607). Microalgae biodiesel was characterized with respect to calorific value, cetane number, density, viscosity, cloud and pour points, water and sediment, flash point, copper strip corrosion, oxidation stability, and acid number according to the ASTM and Indian biodiesel standards (Table 4). Acid number represents the content of free fatty acids in the sample and is indicator of degradation state of biodiesel. Acid number of the produced biodiesel was higher (0.49 mg KOH/g) which was within the limits of biodiesel standards. Higher acid value leads to operational problems, such as formation of deposit in fuel injector and corrosion of storage tank. Density of microalgae biodiesel was 889 kg/m^3 in comparison to *Enteromorpha compressa* algal biodiesel (878.47 kg/m^3) (Suganya et al., 2013). Biodiesel density mainly depends up on its fatty acid ester composition and the remained quantity of alcohol. ASTM D445 method was used for detecting the kinematic viscosity, according to which biodiesel viscosity should be between 1.9 and 6.0 cSt at 40 °C. In the present study, viscosity of microalgae biodiesel was 5.72 cSt which is higher than *Enteromorpha compressa* algal biodiesel (4.35 cSt) (Suganya et al., 2013) but found within ASTM standards. Higher viscosity causes to insufficient fuel atomization leading to incomplete combustion and carbon deposition on the injector and valve seats while lower viscosity is easier to pump and makes final droplets on injector (Sahoo and Das, 2008).

Table 4
Fuel properties of microalgae biodiesel.

S. No.	Parameters	Method	Microalgae biodiesel	Standard limits
1.	Acid number (mg KOH/g)	D664	0.49	0.50 max
2.	Density at 15 °C (kg/m^3)	D7777	889	–
3	Viscosity at 40 °C (mm^2/sec) or cSt	D445	5.72	1.9–6.0
4	Pour point (°C)	D97	−12	–
5	Flash point (°C)	D93	155	>130 min
6	Cetane number		57.03	47 min
7	Copper strip corrosion	D130	1	No.3. max
8	Calorific value (MJ/kg)	D240	39.45	–
9	Water and sediment (%)	D2709	0.03	0.050 max
10	Methyl linolenate (%)	EN 14103	7.54	12%
11	Unsaturated ester (>4 double bonds)%	Internal method-GC	0	1%
12	Oxidation stability (IP, at 140 °C, h)	ASTM-D 7545 & prEN16091	3.08	3 (min)

Flash point represents the flammability of the fuel. Higher flash point favours the safety during handling, transportation, and storage. The flash point of microalgae biodiesel was recorded 155 °C as compared to *Enteromorpha compressa algal* biodiesel (166 °C). Fuel consumption of engine is highly affected by Calorific value. In the current study, microalgae biodiesel has the calorific value of 39.45 MJ/kg which is lower than petroleum diesel (44.24 MJ/kg) but acceptable. Cold properties is the another property that showed biodiesel performance in lower temperature region. Cold properties such as Pour point microalgae biodiesel was observed −12 °C which have better than petroleum diesel (−2 °C). Cetane number is the indicator of ignition quality of fuel which measures combustion behaviour of fuel. In this study, the value of cetane number (CN) of algae biodiesel was found 57.03 which is lesser than *Enteromorpha compressa algal* biodiesel (58.5) but under the limit. High cetane value is the indicator of better combustion, low nitrous oxide (NOx) emission, less occurrence of knocking and easier start-up of engine. Water content observed for *Chlorella vulgaris* was 0.03% which was under the maximum value of 0.05% specified in the ASTM D6751 standard. However, biodiesel is hygroscopic in nature and absorbs moisture from atmosphere which leads to corrosion during storage of biodiesel (Kusdiana and Saka, 2004). Besides, copper corrosion study showed that it was class 1 for microalgae biodiesel, indicating less corrosive for engine parts with time.

Oxidation stability is one of the important properties that describe the degradation tendency of biodiesel. Oxidation of biodiesel is affected by affected by various factors *i.e.* light, unsaturated fatty acids, air, temperature, and presence of traces of metals. Generally, polyunsaturated FAMEs (with more than one double bond) have negative impact on the oxidation stability of biodiesel because they have reactive sites which are susceptible for free radical attack. According to European standards EN14214, an ideal biodiesel should not contain more than 12% and 1% of linolenic (C18:3) and polyunsaturated (having more than 3 double bonds) acids respectively. In this study, linolenic acid contributes 7.54% and 0.07% of microalgal and Jatropha FAME respectively, whereas, polyunsaturated FAME with >4 double bond were found absent in both the cases. It follows ASTM D-6751 standard (minimum 3 h) but does not meet the minimum limit as required by EN-14112 and IS-15607 (6 h). This may be due to the presence of more polyunsaturated FAME in microalgae biodiesel.

4. Conclusion

As conclusion, the present study describes the integrated approach of upstream and downstream processes for microwave

assisted sustainable biodiesel production from microalgae Chlorella vulgaris. The results revealed that Chlorella vulgaris was successfully grown in open raceway pond at semi-continuous mode with biomass productivity of 19.61 g/m^2/day under outdoor conditions. 84.01% biodiesel yield was achieved via two-step microwave assisted acid-base transesterification. Moreover, Chlorella vulgaris is able to produce 13.62 ton/hectare/year biodiesel annually. However, further research is also required to utilize lipid extracted biomass for production of automotive fuels via anaerobic digestion or pyrolysis to make it economically viable at industrial scale.

Acknowledgement

Authors would like to acknowledge the Vivekananda Institute of Algal Technology (VIAT), Chennai (India) to provide *Chlorella vulgaris* strain for present study. Authors are also thankful to the Chancellor Dr. S. J. Chopra, Vice Chancellor Dr. Shrihari and R&D department, University of Petroleum and Energy Studies, Dehradun for their continuous encouragement and valuable mentorship.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.06.013.

References

Adesanya, V.O., Cadena, E., Scott, S.A., Smith, A.G., 2014. Life cycle assessment on microalgal biodiesel production using a hybrid cultivation system. Bioresour. Technol. 163, 343–355.

Anandarajah, K., Mahendraperumal, G., Sommerfeld, M., Hu, Q., 2012. Characterization of microalga *Nannochloropsis* sp. mutants for improved production of biofuels. Appl. Energy 96, 371–377.

Becker, E.W., 1994. In: Baddiley, J. et al. (Eds.), Microalgae: Biotechnology Microbiology. Cambridge Univ. Press, Cambridge (New York).

Bhowmick, G.D., Subramanian, G., Mishra, S., Sen, R., 2014. Raceway pond cultivation of a marine microalgae of Indian origin for biomass and lipid production: a case study. Algal Res. 6, 201–219.

Chen, L., Liu, T., Zhang, W., Chen, X., Wang, J., 2012. Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. Bioresour. Technol. 111, 208–214.

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25, 294–306.

Demirbas, A., 2009. Production of biodiesel from algae oils. Energy Sour. Part A – Recov. Util. Environ. Eff. 31, 163–168.

Granados, M.R., Acién, F.G., Gómez, C., Sevilla, J.M.F., Grima, E.M., 2012. Evaluation of flocculants for the recovery of freshwater microalgae. Bioresour. Technol. 118, 102–110.

Gude, V.G., Patil, P., Guerra, E.M., Deng, S., Nirmalakhandan, N., 2013. Microwave energy potential for biodiesel production. Sust. Chem. Processes 1, 5.

Jaliliannosratia, H., Amina, N.A.S., Kiakalaieha, A.T., Noshadib, I., 2013. Microwave assisted biodiesel production from *Jatropha curcas*L. seed by two-step in situ process: optimization using response surface methodology. Bioresour. Technol. 136, 565–573.

Kang, N.K., Lee, B., Shin, S.E., Jeon, S., Park, M.S., Yang, J.W., 2015. Use of conditioned medium for efficient transformation and cost effective cultivation of *Nannochloropsis salina*. Bioresour. Technol. 181, 231–237.

Kanitkar, A., Balasubramanian, S., Lima, M., Boldor, D., 2011. Acritical comparison of methyl and ethyl esters production from soybean and ricebran oil in the presence of microwaves. Bioresour. Technol. 102 (17), 7896–7902.

Karpagam, R., Raj, K.J., kumar, B.A., Varalakshmi, P., 2015. Characterization and fatty acid profiling in two fresh water microalgae for biodiesel production: lipid enhancement methods and media optimization using response surface methodology. Bioresour. Technol. 188, 177–184.

Khan, S., Rashmi, A., Hussaina, M.Z., Prasad, S., Banerjee, U.C., 2009. Prospects of biodiesel production from microalgae in India. Renew. Sustain. Energy Rev. 13, 2361–2372.

Khudhair, A.M., Farid, M.M., 2011. A review on energy conservation in building applications with thermal storage by latent heat using phase change materials. Energy Convers. Manage. 45 (2), 263–275.

Kim, J., Yoo, G., Lee, H., Lim, J., Kim, K., Kim, C.W., Park, M.S., Yang, J.W., 2013. Methods of downstream processing for the production of biodiesel from microalgae. Biotechnol. Adv. 31 (6), 862–876.

Kumar, K., Mishra, S.K., Shrivastav, A., Park, M.S., Yang, J.W., 2015. Recent trends in the mass cultivation of algae in raceway ponds. Renew. Sustain. Energy Rev. 51, 875–885.

Kusdiana, D., Saka, S., 2004. Effects of water on biodiesel fuel production by supercritical methanol treatment. Bioresour. Technol. 91, 289–295.

Leung, D.Y.C., Guo, Y., 2006. Transesterification of neat and used frying oil: optimization for biodiesel production. Fuel Processing Technol. 87, 883–890.

Mazzocchia, C., Modica, G., Martini, F., Nannicini, R., Venegoni, D., 2004. 2004. Biodiesel and FAME from triglycerides over acid and basic catalysts assisted by microwave. Proceedings of second international conference on microwave and its scientific applications: 2004, vol. 7. CR Chimie, Ancona, Italy, p. 601.

Nigam, P.S., Singh, A., 2010. Production of liquid biofuels from renewable resources. Prog. Energy Combust. Sci. 37, 52–68.

Patil, P., Reddy, H., Muppaneni, T., Ponnusamy, S., Sun, Y., Dailey, P., Cook, P., Patil, U., Deng, S., 2013. Optimization of microwave-enhanced methanolysis o falgal biomass to biodiesel under temperature controlled conditions. Bioresour. Technol. 137, 278–285.

Rashid, N., Rehman, M.S.U., Sadiq, M., Mahmood, T., Han, J.I., 2014. Current status, issues and developments in microalgae derived biodiesel production. Renew. Sustain. Energy Rev. 40, 760–778.

Sahoo, P.K., Das, L.M., 2008. Combustion analysis of Jatropha, Karanja and Polanga based biodiesel as fuel in a diesel engine. Fuel 88, 994–999.

Silitonga, A.S., Ong, H.C., Masjuki, H.H., Mahlia, T.M.I., Chong, W.T., Yusaf, T.F., 2013. Production of biodiesel from *Sterculia foetida* and its process optimization. Fuel 111, 478–484.

Suganya, T., Gandhi, N.N., Renganathan, S., 2013. Production of algal biodiesel from marine macroalgae *Enteromorpha compressa* by two step process: optimization and kinetic study. Bioresour. Technol. 128, 392–400.

Teo, C.L., Idris, A., 2014. Rapid alkali catalyzed transesterification of microalgae lipids to biodiesel using simultaneous cooling and microwave heating and its optimization. Bioresour. Technol. 174, 311–315.

Tiwari, A.K., Kumar, A., Raheman, H., 2007. Biodiesel production from Jatropha oil (*Jatropha curcas*) with high free fatty acids: an optimized process. Biomass Bioenergy 31, 569–575.

Veeramuthu, A.K., Salam, Z., Sathishkumar, P., Hadibarata, T., Yusoff, A.R.M., Ani, F. N., 2015. Exploration of fast growing *Botryococcus sudeticus* for upstream and downstream process in sustainable biofuels production. J. Clean. Prod.

Veeramuthu, A.K., Agila, E., Salam, Z., Ponraj, M., Din, M.F.M., Ani, F.N., 2014a. A study on large scale cultivation of *Microcystis aeruginosa* under open raceway pond at semi-continuous mode for biodiesel production. Bioresour. Technol. 172, 186–193.

Veeramuthu, A.K., Agila, E., Sivakuma, P., Salam, Z., Rengasamy, R., Ani, F.N., 2014b. Optimization and characterization of biodiesel production from microalgae *Botryococcus* grown at semi-continuous system. Energy Convers. Manage. 88, 936–946.