



Production of biodiesel from unused algal biomass in Punjab, India

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Received: 10 February 2017 / Published online: 12 December 2017

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Abstract

Biodiesel from inedible sources has become prominent in last few decades. But it is economically incompatible with petroleum diesel. At the same time, both petro-diesel and biodiesels are concerned with environmental pollution, global warming, etc. Algae, on the other hand, utilize CO₂ for their growth and can minimize some sort of pollution level and results in carbon credit for a country. In Punjab, India, algae are seen to grow in many water bodies. But all those are taken away and dumped in vats. Some of this huge biomass was used for production of biodiesel in this work. Extraction of oil from algae was conducted by using Soxtherm (solvent extraction). An amount of 9 wt% of algal oil was extracted by comparatively costly hexane, whereas 8% extraction was done by cheaper acetone. In the transesterification reaction, molar ratio (methanol: oil) of 6:1, catalyst (KOH) concentration of 3 wt%, reaction temperature of 60 °C, 60 min reaction time and a settling time of 2.5 h were found to be optimum conditions to get maximum ester with minimum free fatty acid content and viscosity. A statistical analysis for the transesterification procedure also showed a methanol-to-oil molar ratio of 6:1 and catalyst concentration of 3 wt% to be the optimum. Characterization of biodiesel was done and compared with ASTM/BIS standards. Most important properties of biodiesel ester like viscosity (3.12 cSt or 3.12 mm²/s), cloud and pour point (−1 and −6 °C, respectively), flash and fire point (153 and 158 °C), carbon residue content (0.03%), acid number (0.36 mg of KOH/gm) were within the range of concerned standards.

Keywords Algae · Biodiesel · Biodiesel properties · Transesterification · Oil extraction

1 Introduction

The future gap, between the worldwide energy demand and its availability, has forecast for decades to be filled by alternate sources. Although some new renewable and non-renewable techniques like wind energy, solar energy, hydro energy and nuclear energy and resources like animal and plant waste, animal fat (Bankovic-Ilic et al. 2014), vegetable oil (Gadge and Raheman 2005; Aicantara et al. 2000; Baladhiya and Joshi 2016; Adewale et al. 2015), which have been proven to be more eco-friendly resources

than petro-diesel (Shirmeshan et al. 2012; Shirmeshan 2013), have become very popular, their high price is becoming an obstacle for them to come into regular use. At the same time, it has been proven that the quantity of biofuels from living sources like vegetable oil, animal fat are not enough to meet world energy requirement for transportation fuel (Smith et al. 2010). On top of that, the conflict of food versus energy (Parman et al. 2011) has been taking place worldwide and it has been reported that the production of biofuel has caused significant increases in the price of regularly used food stuffs like corn and sugar (Seo et al. 2014). So, countries like India (Kumar et al. 2013) which import 43% of their total edible oil in each year cannot produce biodiesel from edible resources from the ethical point of view. Environmental pollution due to conventional and some non-conventional usage in many sectors, especially transport (Conti and Holtberg 2011), has become a threat to many biospheres. So, from these points of view algae are good alternatives for production of bio-fuel which is eco-friendly [fixes 0.6% CO₂ (Ponnusamy et al. 2014)]. Algae lack many drawbacks which are inherited by many other fuel crops. In much research, algal

Edited by Xiu-Qin Zhu

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growth supplemented with carbon in the form of carbon dioxide has shown very good growth along with better lipid content (Holbrook et al. 2014; Dassey et al. 2014; Widjaja et al. 2009). Algae take 100–200 times less than the land area required by oil crops like soya bean, jatropha or palm to grow, while it has 10–20 times more oil yield and much better growth rate (Chisti 2007, 2008; Meng et al. 2009). Moreover, it can be grown on nonproductive land (Costa and Moraise 2011) using variety of water sources. Brackish water, wastewater (Woertz et al. 2009; Christenson and Sims 2011), etc., can be used for its growth (Ashokkumar et al. 2014). Although biofuel from algae, especially biodiesel, is not a very new technology, its use is still confined mostly to laboratories mainly for its high oil price and unawareness about the importance of algae as a fuel resource. In most cases, algae, for biodiesel production, are grown in photobioreactors (Podevin et al. 2017) to avoid contamination and increase lipid content in artificially controlled environment. This technology is very costly (Nosker et al. 2011) and causes increases in oil price (Mata et al. 2009). Supply of freshwater, which is a mandatory option for algae culture in these cases, is also not an environment-friendly concept (Chisti 2013). In Punjab, India, algal bloom takes place rapidly in many water bodies including cattle ponds, canals and pisciculture ponds. This huge quantity of algae remains unused and causes pollution in those water bodies by making a film at the surface and not allowing the sun light to penetrate the water. Therefore, those algae are thrown away in vats. In this experiment, these unused algae were collected from some specific sites and used to produce biodiesel to examine their efficiency to produce energy. As the main raw material algae used in this case had no commercial value, it was assumed that the algal oil and thereby algal biodiesel produced in this work would be cheaper in price.

2 Materials and method

2.1 Selection and collection of algal biomass

Algal biomass was primarily collected from ponds which are used for pisciculture at Punjab Agricultural University campus, Punjab, India. In these ponds, culture and experiments on commercial fish take place throughout the year. These ponds get cleaned in winter. Huge algal blooms take place in these ponds at this time. These algae are thrown away to re-prepare the ponds. Some specimens of algae were collected from Simlapuri Nahar (canal) of Ludhiana, Punjab. These algae, collected from these two sites, were chosen for the experiment as they are (1) economically unimportant, (2) easily available and (3) not used by any one for any purpose. Collection of algae was done by a

simple algae net, and these algae were brought to the laboratory for drying.

2.2 Identification of algal species

Sample of algae collected from all ponds underwent microscopic examination for the identification of their species. All samples were examined at 10 \times , 40 \times and 100 \times magnifications. Species were identified according to their morphological characters.

2.3 Drying of algae

To make this process eco-friendly and economically cheap, all collected algae were divided to small chunks and partially dewatered manually by pressing. These were kept in the sun on the roof top for 2–3 days for complete drying. The daytime temperature of the area varied from 7 to 12 °C at that time. As in summer days (day temperature 30–45 °C), keeping algae in the sun for 1–1.5 days was found to be enough for complete drying, use of electric oven for drying was completely avoided.

2.4 Oil extraction

Dried algal biomass was ground to powder (Fig. 1) by a mixer grinder and stored in dry, airtight glass vessels. Solvent extraction was used for extracting oil from this algae powder. Digital Soxtherm apparatus (Gerhardt) (Fernández et al. 2010; Silva et al. 2011) was used for conducting this part of the experiment. Oil from algae was extracted by using hexane and acetone. Algae (5 gm) were taken in each Soxtherm-thimble. The weights of the collection vessels were taken at the beginning and after 2–3 repetitions of the oil extraction procedure. After extraction, oil was collected in glass vessels for further experiment. Excess solvents were collected by Soxtherm in the collection tank. The solvents were recovered for further



Fig. 1 Dried algae and its powder

experiments. Recovery of 80%–92% of solvents was done by this machine which helped to stop the wastage of solvents and keep the whole process cheap.

2.5 Biodiesel production from algal oil

Biodiesel was produced from crude algal oil by transesterification. The free fatty acid (FFA) content of the crude oil was measured before transesterification. FFA of the oil was 21.3%. As the FFA was very high, a two-step esterification procedure, i.e., acid esterification followed by a conventional base esterification or transesterification in other word (Sorguven and Ozilgen 2010) was performed (Dhawane et al. 2016; Suganya et al. 2013; Berchmans and Hiratab 2008). Different parameters like alcohol (methanol)-to-oil molar ratio (3:1; 4:1; 5:1; 6:1, 7:1, 8:1 for both acid esterification and alkaline esterification), catalyst amount (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 wt%), reaction time (30, 45, 60, 75, 90, 105 and 120 min), reaction temperature (40, 45, 50, 55, 60 and 62 °C) and settling time (60, 90, 120, 150, 180, 210, 240, 270 and 300 min) were used to optimize the procedure. Potassium hydroxide (KOH) was used as a base catalyst, and concentrated sulfuric acid (conc. H_2SO_4) was used as an acid catalyst in this experiment. All the acid esterification and transesterification reactions were done in a water bath shaker. The reactants were put inside screw-capped conical flasks, and the flasks were fastened in the slots of the water bath shaker (Ragit et al. 2011). The conical flasks were stirred at 250 rpm (Musa 2016) inside the water bath at a preset constant temperature for the reaction to take place inside the conical flask. When one parameter was being optimized, all other parameters were kept constant and the optimized parameters were kept constant at the time of optimization of next parameters. Just after the transesterification reaction, the solution was put in a separating funnel to separate glycerol. The settling time for complete separation of glycerol from the ester was also optimized. The final product was washed with hot distilled water followed by heating at 100 °C in the oven to remove water and get crystal clear biodiesel. The flowchart of this experiment is given in Fig. 2.

Besides this practical experiment (non-statistical), a statistical analysis for the optimization of transesterification procedure was performed using the Taguchi method (Ross 1989; Buasri et al. 2009). Four factors namely molar ratio (X_1), catalyst concentration (X_2), reaction time (X_3) and reaction temperature (X_4) were considered as variables for this analysis (Table 3).

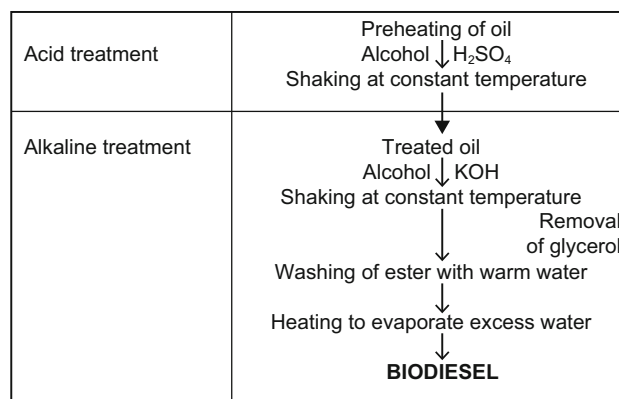


Fig. 2 Flowchart of acid and alkaline treatment of oil for biodiesel production

2.6 Fuel characterization

Produced biodiesel was stored in closed airtight dry glass vessels. Different characteristics (Ragit et al. 2012) of biodiesel were tested by standard methods (Table 1) to check its eco-friendliness and suitability in a CI engine. All characters of the biodiesel were tallied with BIS/ASTM standards.

3 Result and discussion

3.1 Identified algal species

In the mixed indigenous culture of algae collected from PAU, ten species were found to be present. The identified species were *Chlorella* sp., *Closterium* sp., *Ocellularia* sp., *Spirulina* sp., *Navicula* sp., *Pinularia* sp., *Zygnema* sp., *Spyrogyra* sp., *Scenedesmus* sp., *Gomphonema* sp.

In Simlapuri Nahar, *Euglena* sp., *Spirulina* sp., *Chlorella* sp., *Spyrogyra* sp. *Urenoma* sp., *Frustulia* sp., were present.

3.2 Weight of dried algal biomass

A huge reduction in the weight of algal species took place after the drying of algae. The weight of fully dried algal mass was found to be one-tenth of the primary weight. After complete dewatering, 1–1.5 kg of dried algae was obtained from every 10 kg of wet algae.

3.3 Yield of algal oil

Yield of the oil was calculated as the fraction of weight of the oil produced per kilogram of dried algal mass from which the oil was extracted (Oil yield = $\frac{\text{Oil extracted}}{\text{Dried algal biomass taken}} \times 100$).

It was found that the oil extraction capacity of hexane is the

Table 1 Properties and standard methods for fuel characterization

S. no.	Fuel property	Testing apparatus	Standard	Maker
1	Relative density	Pycnometer	IS: 1448 [P:32]: 1992	Borosil
2	Kinematic viscosity	Redwood viscometer	IS: 1448 [P:25] 1976	Widsons
3	Gross heat of combustion	Digital bomb calorimeter	IS: 1448 [P:6]: 1984	Widsons
4	Cloud and pour point	Cloud and pour point apparatus	IS: 1448 [P:10]: 1970	Widsons
5	Flash and fire point	Flash and fire point apparatus	IS: 1448 [P:32]: 1992	Widsons
6	Carbon residue	Carbon residue content apparatus	ASTM D189–IP 13 of institute of petroleum	Widsons
7	Ash content	Muffle furnace	ASTM D482-IP 4 of Institute	Popular scientific

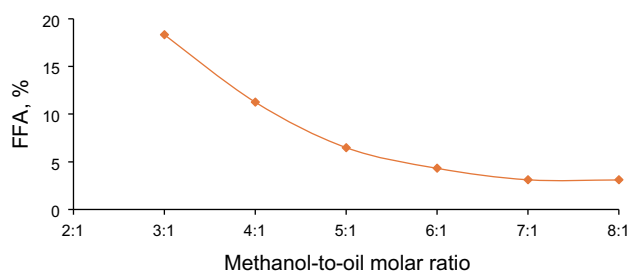
higher (9%) of the two solvents. The acetone was able to extract almost 8.5% of the algal oil. For the rest of the experiment, acetone was used to extract oil as it is much cheaper than hexane.

3.4 Optimization of biodiesel production process

3.4.1 Acid treatment

Acid treatment was done to decrease the FFA of algal oil to a certain level from where transesterification can be initiated. This step also helped to remove the impurities present in the oil.

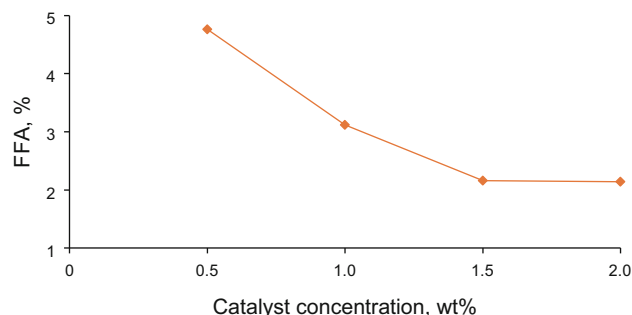
3.4.1.1 Optimization of methanol-to-oil molar ratio Six methanol-to-oil molar ratios (3:1, 4:1, 5:1, 6:1, 7:1, 8:1) were compared in a row. At this time, all other parameters were kept constant (catalyst concentration of 2 wt%; reaction temperature of 55 °C; reaction time of 75 min). There was a huge change in FFA while the methanol-to-oil molar ratio increased from 3:1 to 6:1. At molar ratio of 7:1, the FFA of the oil was reduced to 3.12%. No remarkable change of FFA was observed by further increasing the molar ratio (Fig. 3). In each of the next optimization experiments in acid esterification, a methanol-to-oil molar ratio of 7:1 was used.

**Fig. 3** Optimization of methanol-to-oil molar ratio (acid esterification)

3.4.1.2 Optimization of catalyst concentration Four catalyst concentrations (0.5, 1.0, 1.5 and 2.0 wt%) were taken for the optimization of acid treatment procedure. The FFA content of the algal oil dropped down to 1.83% when 1.5 wt% of catalyst was used. No further decrease was found by using more H₂SO₄ (Fig. 4). In the next experiments of acid esterification, methanol to oil molar ratio of 7:1 and catalyst concentration of 1.5% were taken.

3.4.1.3 Optimization of reaction temperature Among six reaction temperatures, the lowest FFA was seen at 55 °C. But at the same time, it gave exactly same FFA (1.72%) when the reaction was done at 60 °C. So, at the time of optimization of reaction time, both reaction temperatures (55 and 60 °C) were observed and examined again with all reaction times along with previously optimized parameters (molar ratio and catalyst concentration) (Fig. 5).

3.4.1.4 Optimization of reaction time It was found that 90 min was enough to complete the reaction. The FFA dropped to 1.06% (55 °C) and 1.09% (60 °C). No further significant change was found by increasing the time any further (Fig. 6). Although there was a very minute difference between the FFA values derived from the esters produced at 55 and 60 °C, the former one was taken as the optimized parameter for forthcoming reactions (Table 2).

**Fig. 4** Optimization of acid catalyst concentration (acid esterification)

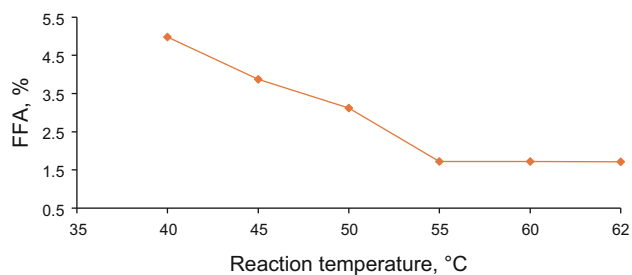


Fig. 5 Optimization of reaction temperature (acid esterification)

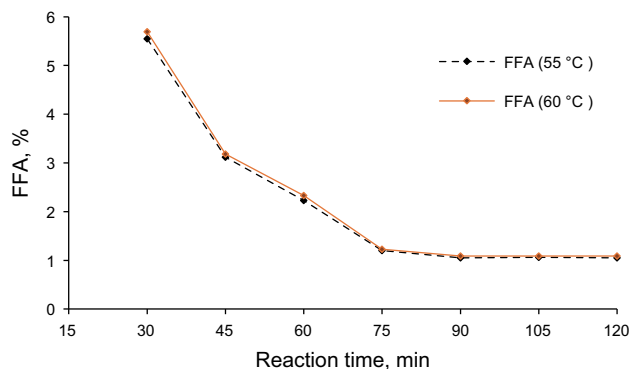


Fig. 6 Optimization of reaction time (acid esterification)

Table 2 Optimized parameters of algal biodiesel production

Acid treatment	
Methanol-to-oil molar ratio	7:1
Catalyst (H ₂ SO ₄) concentration	1.5 wt%
Reaction temperature	55 °C
Reaction time	90 min
Settling time	180 min
Alkaline treatment	
Methanol-to-oil molar ratio	6:1
Catalyst (KOH) concentration	3 wt%
Reaction temperature	60 °C
Reaction time	60 min
Settling time	150 min

3.4.1.5 Optimization of settling time Ester produced in the acid esterification reaction was settled completely after 3 h (180 min). Two completely separate phases were appeared. The ester was separated and used in the optimization of transesterification process.

3.4.2 Alkaline treatment (transesterification)

Transesterification reaction for biodiesel production takes place as a result of exchange of organic R group of

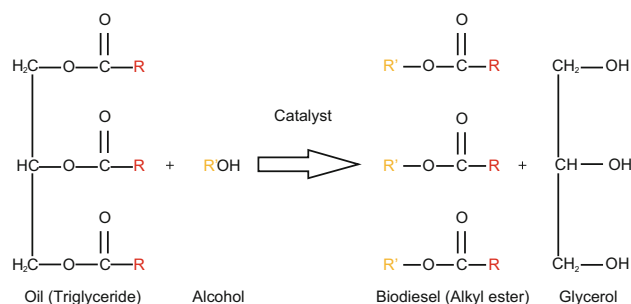


Fig. 7 Transesterification reaction

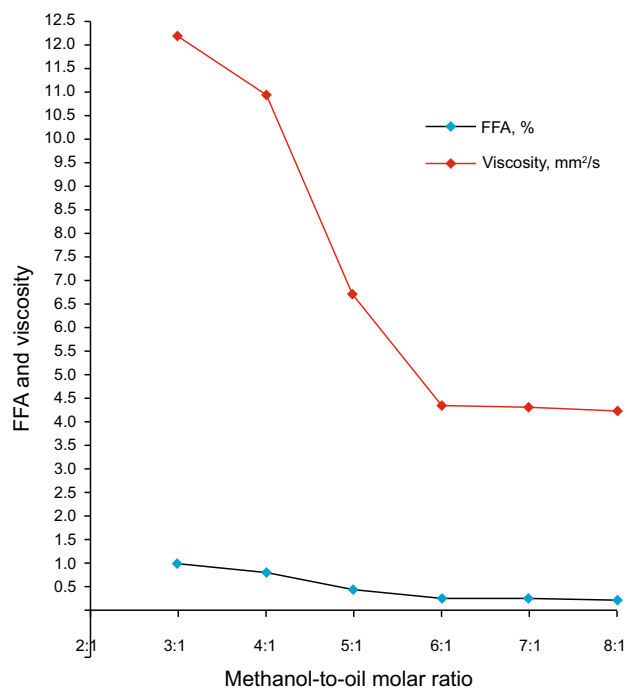


Fig. 8 Optimization of methanol-to-oil molar ratio

particular oil with an R' group of an alcohol (Fig. 7). In this experiment, FFA along with the viscosity of biodiesel was considered as the yardsticks to optimize the procedure as viscosity plays a major role to carry out the functions and the performance of the CI engine.

3.4.2.1 Optimization of methanol-to-oil molar ratio Here, again all the six methanol-to-oil molar ratios (3:1–8:1) were applied. The FFA of the biodiesel started decreasing for the application of 3:1 molar ratio and reached almost its lowest value (0.25%) when 6:1 molar ratio was applied. The viscosity of the ester was also almost lowest (4.35 mm²/s) at 6:1 this time. No further increase in molar ratio could cause any effective change in FFA content and viscosity (Fig. 8).

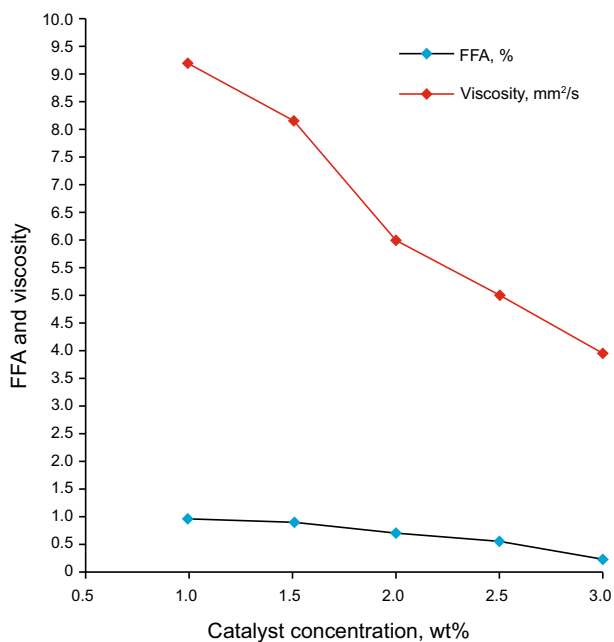


Fig. 9 Optimization of catalyst concentration

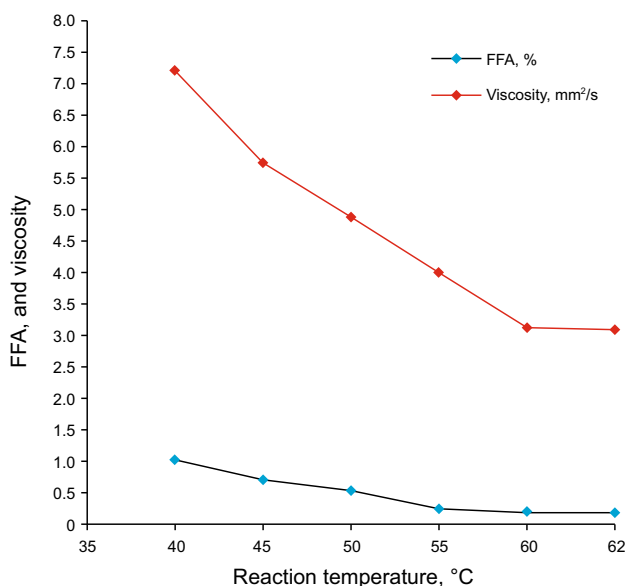


Fig. 10 Optimization of reaction temperature

3.4.2.2 Optimization of catalyst concentration Catalyst concentration was varied from 1.0 wt% to 3.5 wt%. The FFA came down to 0.21% while 3 wt% of catalyst and methanol-to-oil molar ratio of 6:1 were used. Using 3.5 wt% of catalyst resulted in no cut down in FFA of the ester, but formation of soap. The repetition of the experiment with same catalyst concentration yielded same result. So, KOH of 3.5 wt% was not considered in this experiment anymore and FFA along with the viscosity of the same was not calculated. So, the catalyst concentration of 3 wt% was

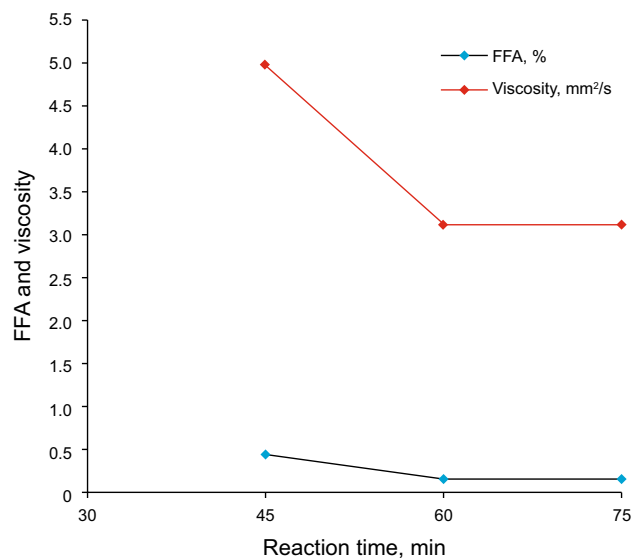


Fig. 11 Optimization of reaction time

noted as the optimum catalyst concentration for transesterification (Fig. 9). The viscosity of the ester was 3.96 mm²/s at this time.

3.4.2.3 Optimization of reaction temperature A steep fall of FFA and viscosity of the biodiesel was found while the reaction temperature increased from 40 °C to 62 °C. A reasonable change in FFA content (0.18%) and viscosity (3.12 mm²/s) was found by increasing the temperature up to 60 °C. A negligible decrease in FFA and viscosity was observed while the transesterification was done at 62 °C (Fig. 10). Therefore, 60 °C was considered as the optimum value of reaction temperature and used for optimizing the reaction time along with methanol-to-oil molar ratio of 6:1 and catalyst (KOH) concentration of 3 wt%.

3.4.2.4 Optimization of reaction time Soap formation (Deng et al. 2010) was found when transesterification was done for 30 min. Hence, the reaction was repeated to confirm the first result. When the result was the same, the reaction time of 30 min was also not considered any more in this experiment. The best result was yielded when the reactants were reacted for 60 min. The FFA of ester was 0.18%, and the viscosity of the same was 3.12 mm²/s after this reaction (Fig. 11). As the FFA did not change by increasing the time of reaction to 75 min, 60 min was considered as the optimum time by considering the concept of energy saving (Table 2).

3.4.2.5 Optimization of settling time It took 150 min to deposit all the glycerol produced at the bottom of the separating funnel. After this, the glycerol was separated and the biodiesel was water washed to remove KOH. A 96.4% biodiesel was obtained from the procedure, and the

presence of fatty acid methyl ester (FAME) was confirmed by using NMR (nuclear magnetic resonance) technique.

3.5 Statistical analysis

To set the levels of the statistical analysis, the appropriate upper and the lower values (7:1 and 5:1 for methanol-to-oil molar ratio, 3.5 and 2.5 wt% for catalyst concentration, 75 and 45 min for reaction time, 62 and 55 °C for reaction temperature) of the optimized values (derived from the non-statistical experiment) along with optimized values (methanol-to-oil molar ratio of 6:1, catalyst concentration of 3 wt%, reaction time of 60 min and reaction temperature of 60 °C) of all these parameters itself were used (Table 3).

L9 orthogonal array was used for designing the experiments to see the effect of four parameters to achieve the highest yield of biodiesel along with lowest free fatty acid content (Table 4). Experiments were conducted again to observe the yield and the FFA content of algal biodiesel resulted for all nine sets of parameters provided by the Taguchi method. Three trials of both yield (%) and FFA (%) of the biodiesel were performed, and their results were noted. Corresponding means of the trials were calculated thereafter.

Table 3 Process parameters taken and their levels

Process parameters	Code	Levels		
Methanol-to-oil molar ratio	X1	5	6	7
Catalyst concentration, wt%	X2	2.5	3.0	3.5
Reaction time, min	X3	45	60	75
Reaction temperature, °C	X4	55	60	62

Table 4 Yield and FFA of the algal biodiesel resulted using orthogonal L9 array and their means (X = parameters; T = trials; Y = mean of corresponding trials)

S. no.	Levels				Yield of biodiesel, %			FFA of biodiesel, %			Mean yield of biodiesel, % Y1	Mean FFA of biodiesel, % Y2
	X1	X2	X3	X4	T1	T2	T3	T1	T2	T3		
1	5	2.5	45	55	70.23	73.88	71.35	0.37	0.36	0.36	71.82	0.36
2	5	3.0	60	60	20.71	18.59	19.87	0.78	0.80	0.83	19.72	0.80
3	5	3.5	75	62	10.12	9.58	9.99	0.97	1.09	1.04	9.90	1.03
4	6	2.5	60	62	92.01	88.97	91.45	0.25	0.23	0.25	90.81	0.24
5	6	3.0	75	55	94.65	93.98	92.53	0.20	0.21	0.19	93.72	0.20
6	6	3.5	45	60	23.86	22.80	24.00	0.59	0.57	0.56	23.55	0.57
7	7	2.5	75	60	82.40	82.02	83.12	0.82	0.81	0.83	82.51	0.82
8	7	3.0	45	62	86.73	88.32	87.86	0.79	0.77	0.80	87.64	0.79
9	7	3.5	60	55	5.20	7.33	5.70	1.11	1.15	1.13	6.08	1.13

3.5.1 Effect of parameters

The yield and FFA of the biodiesel were plotted for nine experimental runs. The signal-to-noise ratio (SNR) achieved from the experiment is plotted in Table 5. The ranks in the table indicate the effect of the parameters on two factors, i.e., the yield and FFA content of algal biodiesel. As our goal was to achieve biodiesel with highest yield and lowest FFA content, the ‘larger is better’ model (SNR) was used for Y1 (yield) and the ‘smaller is better’ for Y2 (FFA).

The ranks indicated that ‘catalyst concentration (X2)’ was the foremost influencing factor for the highest yield and the ‘molar ratio (X1)’ affected the FFA content of the biodiesel the most in comparison with other parameters.

In Fig. 12, the contribution of four parameters to the yield (Fig. 12a) and FFA (Fig. 12b) of biodiesel is shown where higher signal-to-noise ratio shows better effect of that parameter and the optimum level is indicated by the maximum value of SNR in all the graphs. According to the graphs, the optimum level of a parameter for highest biodiesel yield was X1 (methanol-to-oil molar ratio) at 6:1, X2 (catalyst concentration) at 2.5 wt%, X3 (reaction time) at 45 min and X4 (reaction temperature) at 62 °C. The optimum level of a parameter for lowest FFA was X1 at 6:1, X2 at 2.5%, X3 at 45 min, X4 at 55 °C.

3.5.2 Analysis of variance by ANOVA

The significance of parameters was calculated by the percentage of contribution of those parameters on the yield (Y1) of biodiesel as well as the FFA content (Y2) of it (Table 6). The highest contribution (67.7%) for biodiesel yield was due to catalyst concentration (X2), whereas molar ratio (X1) was the second most contributing (17.3%) parameter which was followed by reaction time (X3) and

Table 5 Signal-to-noise ratio for process parameters at different levels

Level	S/N ratio corresponding to Y1				S/N ratio corresponding to Y2			
	X1	X2	X3	X4	X1	X2	X3	X4
1	27.65	38.21	34.47	30.75	3.52	7.67	5.27	7.26
2	35.35	34.73	26.91	30.56	10.42	5.99	4.42	2.85
3	30.95	21.01	32.56	32.64	0.90	1.19	5.15	4.73
Range	7.70	17.20	7.56	2.09	9.52	6.48	0.84	4.42
Rank	2	1	3	4	1	2	4	3

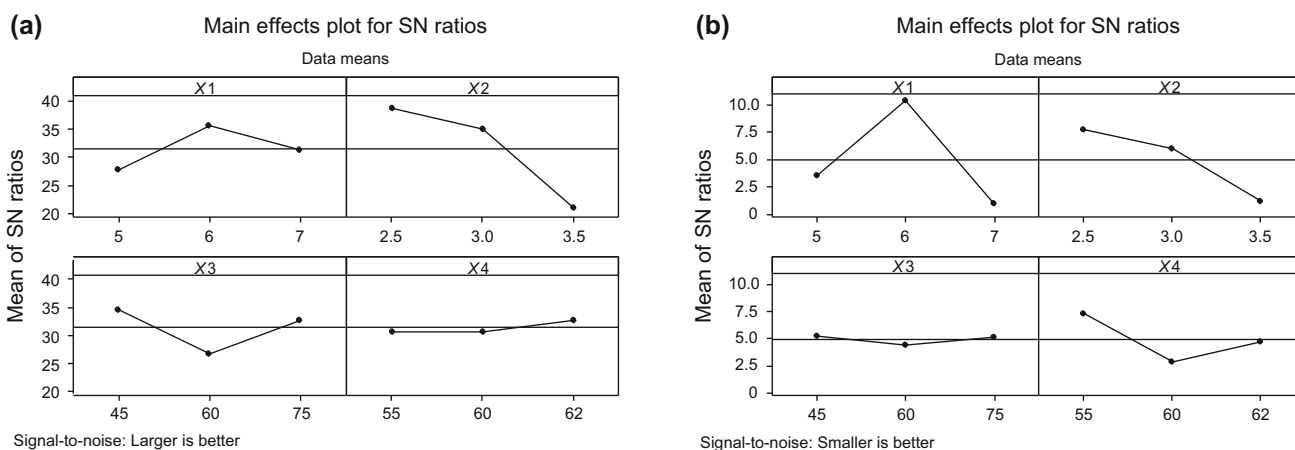


Fig. 12 SNR for each parameter (X1—methanol-to-oil molar ratio; X2—catalyst concentration; X3—reaction time; X4—reaction temperature) for **a** yield, %, and **b** FFA

reaction temperature (X4) providing contribution of 8.9% and 6.1%, respectively. On the other hand, molar ratio with 58.3% contribution was the most significant parameter for lowest FFA. Among the rest of parameters, catalyst concentration (33.7%), reaction temperature (4.5%) and reaction time (3.4%) were significant consecutively.

The equation of the mathematical model below stands for regression analysis of observed data of biodiesel yield (Y1):

$$Y1 = 179 + 12.5X1 - 68.5X2 + 0.035X3 + 0.06X4,$$

$$R^2 = 0.69.$$

To find the effect of parameters (X) on factor Y1 and factor Y2, six 3D graphical plots were prepared using the three most contributing parameters (catalyst concentration, methanol-to-oil molar ratio and reaction time for the highest yield of the biodiesel; molar ratio, catalyst concentration and reaction temperature for the lowest FFA content of algal biodiesel) derived from Table 5 and Fig. 12a, b. The best yield of biodiesel was found to be achieved when the methanol-to-oil molar ratio, catalyst concentration and reaction time increased from 5:1, 2.5 wt% and 60 min to 6:1, 3 wt% and 75 min (Fig. 13). From the previous tables, it has become evident that catalyst concentration was the most contributing parameter for

yielding highest amount of biodiesel. So, it can also be observed from the 3D graph that the highest yield of biodiesel is plotted at 3 wt% catalyst concentration and increase in its amount causes significant cut down in the yield. Following the same procedure, 3D plots for FFA were also prepared using three parameters (methanol-to-oil molar ratio, catalyst concentration, reaction temperature consecutively) which exerted most contribution. The least FFA was seen at methanol-to-oil molar ratio of 6:1, catalyst concentration of 3 wt% and reaction temperature of 55 °C.

3.6 Biodiesel characteristics

All characteristics of algal biodiesel were almost equally good or better than conventional diesel fuel. Moreover, all of them were within the limit of ASTM/ BIS standards (Table 7) (McCurdy et al. 2014; Diesel Net Technology Guide 2009). The kinematic viscosity of the biodiesel was just a little higher than the petroleum diesel (3 mm²/s). At the same time, the acid value of biodiesel was also very low (0.36%) which indicates the chance of corrosion of the parts of engine is less in this case. The ash content and carbon residue content were also very low (0.01% and 0.03%, respectively), and thus the fuel results in very low deposition of carbon in the engine which is good for the

Table 6 Percentage of contribution of parameters

Factor	Process parameter	Sum of square	Contribution, %
For Y1	X1	1997.8	17.31
	X2	7812.8	67.71
	X3	1028.0	8.91
	X4	699.6	6.06
For Y2	X1	0.52	58.43
	X2	0.30	33.71
	X3	0.03	3.37
	X4	0.04	4.49

engine as well as the environment. Therefore, it indicates that the fuel produced can be used in the CI engines with very nominal modifications of the engine. As the flash point of the biodiesel produced from algae was very much higher than that of fossil fuel diesel (54–96 °C), it is much safer than fossil fuel diesel because it decreases the chance of fire hazards many folds (Lee and Ha 2003). Although the calorific value of the biodiesel (40,882 kJ/kg) is lower than that of conventional petroleum diesel (44,800 kJ/kg), it is much higher than that of the biodiesel produced from commonly used feed stocks like palm oil (36,764 kJ/kg) and Jatropha (39,300 kJ/kg). On top of all these, lower

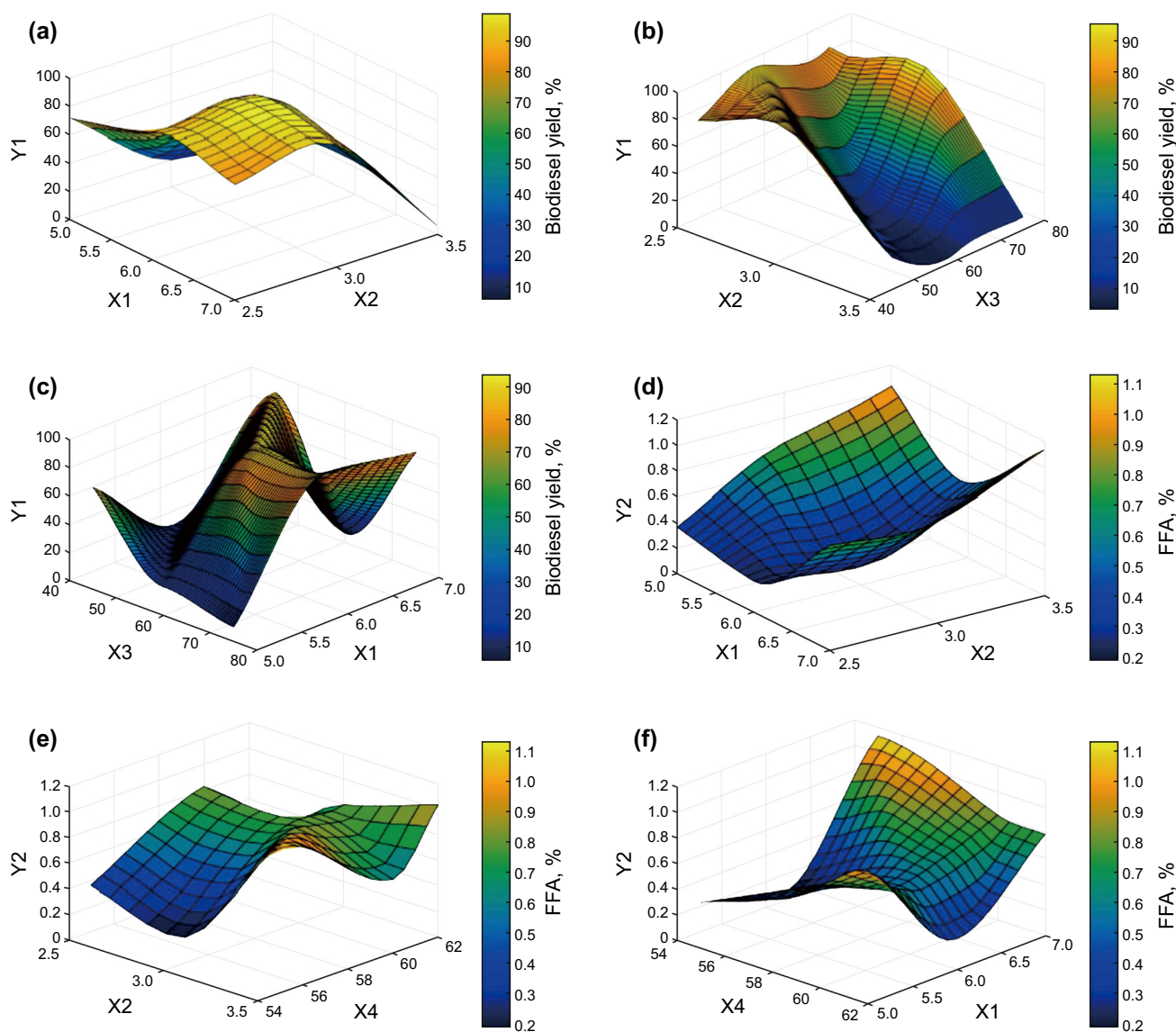


Fig. 13 3D plots of yield of biodiesel with respect to **a** methanol-to-oil molar ratio (X_1) and catalyst concentration (X_2), **b** catalyst concentration (X_2) and reaction time (X_3), **c** reaction time (X_3) and methanol-to-oil molar ratio (X_1); and FFA with respect to **d** methanol-to-oil molar ratio (X_1) and catalyst concentration (X_2), **e** catalyst concentration (X_2) and reaction temperature (X_4), **f** reaction temperature (X_4) and methanol-to-oil molar ratio (X_1)

Table 7 Algal biodiesel characteristics along with ASTM/ BIS standards

Properties	Algal biodiesel	ASTM/BIS standards
Kinematic viscosity at 40 °C, mm ² /s	3.12	1.9–6
Density at 15 °C, kg/m ³	872.9	860–900
Flash point, °C	153	93 (minimum)
Fire point, °C	158	–
Cloud point, °C	– 1	3 (maximum)
Pour point, °C	– 6	–
Acid number, mg KOH/g	0.36	0.50 (maximum)
Ash content, %	0.01	0.01 (maximum)
Carbon residue content, %	0.03	0.050 (maximum)
Calorific value, kJ/kg	40,882	–

cloud point and pour point (–1 and –6 °C) would let it to be used in winter with no modification of engine or addition of additive (Sarin et al. 2009).

4 Conclusion and future research scope

As the diesel engine plays an important role with its high thermal efficiency and low price in energy sector (An et al. 2012), supply of sufficient non-renewable petro-diesel or substitutions of petro-diesel in today's scenario is very important. Algae from that perspective are very promising resource for the production of biodiesel. In this experiment, one of the biofuels of algae, i.e., biodiesel produced, can be a good alternative fuel as it solves the problem of solid waste disposal on the one hand and the problem of environmental pollution on the other.

The 'non-statistical approach' was used to set the levels of the parameters for 'statistical analysis' in this experiment as no research work was done before on this particular culture of feedstock of unused indigenous mixed culture of algae. Although the results derived from the two approaches were different, there was a least difference in the yield of biodiesel produced using both approaches and both were quite satisfactory (>90%). The little difference took place because of the different sets of levels of parameters which were set by the software. As the yield of the biodiesel was good enough (96% obtained from the non-statistical method and 93% obtained from the statistical method) and properties of the same were within the limits of ASTM or BIS standards and were almost similar to petro-diesel, only minor modifications will be required in the engine to run it with B100 (100% biodiesel). It can be assumed that blending of less than 30% will not require any modification of the engine parts. Moreover, the properties of this biodiesel were better than many other commercially used biodiesels. So, this fuel can replace all those biodiesels and create a very good industry and business market. At the time when the economic feasibility of all

biofuels including biodiesel from algae is under question mark (Amano-Boadu et al. 2014), this completely unused mixed culture of algae can be used to produce biodiesel as the feed stock in this case has no price value. Since the acid value and the FFA content of this algal oil are very high, it was mandatory for us to go for a two-step biodiesel production process. But more research in future can help produce biodiesel in single step from this mixed culture of algae. The only constraint in that case would be the oil content of these algae which is comparatively lower than that can be yielded from monocultures of some algal species. So, experiments can be done on the culture of these algae to increase its oil content and the growth rate. Experiments with modern approach like making the procedure carbon neutral by providing the energy of oil extraction and biodiesel production using solar power (Taylor et al. 2013) have been proven in previous experiments. Similar experiments using this feedstock can be performed on these algae which may make the price of the final product reasonably cheaper.

Acknowledgement The authors are thankful to all the members of Thapar University and CSIR CMERI for providing the funding and the laboratory facilities.

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References

- Adewale P, Dumont M, Ngadi M. Recent trends of biodiesel production from animal fat wastes and associated production techniques. *Renew Sustain Rev.* 2015;45:574–88. <https://doi.org/10.1016/j.rser.2015.02.039>.
- Aicantara R, Amores J, Canoira L, et al. Catalytic production of biodiesel from soyabean oil, used frying oil and tallow. *Biomass*

- Bioenergy. 2000;18:515–27. [https://doi.org/10.1016/s0961-9534\(00\)00014-3](https://doi.org/10.1016/s0961-9534(00)00014-3).
- Amano-Boadu V, Pfromm PH, Nelson R. Economic feasibility of algal biodiesel under alternative public policies. *Renew Energy*. 2014;67:136–42. <https://doi.org/10.1016/j.renene.2013.11.029>.
- An H, Wang WM, Chou SK, et al. Combustion of emissions characteristics of diesel engine fuelled by biodiesel at partial load conditions. *Appl Energy*. 2012;99:363–71. <https://doi.org/10.1016/j.apenergy.2012.05.049>.
- Ashokkumar V, Agila E, Salam Z, et al. A study on large scale cultivation of *Microcystis aeruginosa* under open raceway pond at semi-continuous mode for biodiesel production. *Biores Technol*. 2014;172:186–93. <https://doi.org/10.1016/j.biortech.2014.08.100>.
- Baladhiya CS, Joshi DC. Effect of castor cake on biogas production by adding with cattle dung. *Int J Sci Environ Technol*. 2016; 5:547–51.
- Bankovic-Ilic IB, Stojkovic IJ, Stamenkovic OS, et al. Waste animal fats as feedstocks for biodiesel production. *Renew Sustain Rev*. 2014;32:238–54. <https://doi.org/10.1016/j.rser.2014.01.038>.
- Berchmans HJ, Hiratab S. Biodiesel production from crude *Jatropha curcas* L. seed oil with a high content of free fatty acids. *Biores Technol*. 2008;99(6):1716–21. <https://doi.org/10.1016/j.biortech.2007.03.051>.
- Buasri A, Chaiyut N, Katlekha P, et al. Biodiesel production from crude palm oil with a high content of free fatty acids and fuel properties. *Chiang Mai Univ J Nat Sci*. 2009;8:115–24.
- Chisti Y. Constraints to commercialization of algal fuels. *J Biotechnol*. 2013;167(3):201–14. <https://doi.org/10.1016/j.jbiotec.2013.07.020>.
- Chisti Y. Biodiesel from microalgae beats bioethanol. *Trends Biotechnol*. 2008;26:126–31. <https://doi.org/10.1016/j.tibtech.2007.12.002>.
- Chisti Y. Biodiesel from microalgae. *Biotechnol Adv*. 2007;25: 294–306. <https://doi.org/10.1016/j.biotechadv.2007.02.001>.
- Christenson L, Sims R. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnol Adv*. 2011;29:686–702. <https://doi.org/10.1016/j.biotechadv.2011.05.015>.
- Conti J, Holtberg P. International energy outlook. Technology report. 2011. www.eia.gov.
- Costa JAV, Moraise MG. The role of biochemical engineering in the production of biofuels from microalgae. *Biores Technol*. 2011;102:2–9. <https://doi.org/10.1016/j.biortech.2010.06.014>.
- Dassey AJ, Hall SG, Theegla CS. An analysis of energy consumption for algal biodiesel production: comparing the literature with current estimates. *Algal Res*. 2014;4:89–95. <https://doi.org/10.1016/j.algal.2013.12.006>.
- Deng X, Fang Z, Liu YH. Ultrasonic transesterification of *Jatropha curcas* L. oil to biodiesel by a two-step process. *Energy Convers Manag*. 2010;51:2802–7. <https://doi.org/10.1016/j.enconman.2010.06.017>.
- Dhawane SH, Kumar T, Halder G. Biodiesel synthesis from *Hevea brasiliensis* oil employing carbon supported heterogeneous catalyst: optimization by Taguchi method. *Renew Energy*. 2016;89:506–14. <https://doi.org/10.1016/j.renene.2015.12.027>.
- Fernández CM, Ramos MJ, Perez A, et al. Production of biodiesel from winery waste: extraction, refining and transesterification of grape seed oil. *Biores Technol*. 2010;101:7019–24. <https://doi.org/10.1016/j.biortech.2010.04.014>.
- Gadge SV, Raheman H. Process optimization for biodiesel production from mahua (*Madhuca indica*) oil for using response surface methodology. *Biores Technol*. 2005;97:379–84. <https://doi.org/10.1016/j.biortech.2005.03.014>.
- Holbrook GP, Davidson Z, Tatara RA, et al. Use of microalga *Monoraphidium* sp. grown in waste water as a feedstock for biodiesel: cultivation and fuel characterization. *Appl Energy*. 2014;131:386–93. <https://doi.org/10.1016/j.apenergy.2014.06.043>.
- Kumar M, Sharma MP, Dwivedi G. Algae oil as future energy source in Indian perspective. *Int J Renew Energy Res*. 2013;3(4): 914–21.
- Lee S, Ha D. The lower flash points of binary systems containing non-flammable component. *Korean J Chem Eng*. 2003;20:799–802. <https://doi.org/10.1007/bf02697279>.
- Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel productions and other applications: a review. *Renew Sustain Energy Rev*. 2009;14:217–32. <https://doi.org/10.1016/j.rser.2009.07.020>.
- McCurdy AT, Higham AJ, Morgan MR, et al. Two-step process for production of biodiesel blends from oleaginous yeast and microalgae. *Fuel*. 2014;137:269–76. <https://doi.org/10.1016/j.fuel.2014.07.099>.
- Meng X, Yang J, Xu X, et al. Biodiesel production from oleaginous microorganisms. *Renew Energy*. 2009;34:1–5. <https://doi.org/10.1016/j.renene.2008.04.014>.
- Musa IA. The effects of alcohol to oil molar ratios and the type of alcohol on biodiesel production using transesterification process. *Egypt J Pet*. 2016;25:21–31. <https://doi.org/10.1016/j.ejpe.2015.06.007>.
- Nosker NH, Barbosa MJ, Vermue MH, et al. Microalgal production— a close look at the economics. *Biotechnol Adv*. 2011;29:24–7. <https://doi.org/10.1016/j.biotechadv.2010.08.005>.
- Parman B, Amanor-Boadu V, Pfromm P, et al. Third generation biofuels and the food vs fuel debate: a systems perspective. *Int J Environ Cult Econ Soc Sustain*. 2011;7:287–300. <https://doi.org/10.18848/1832-2077/cgp/v07i02/54905>.
- Podevin M, Fotidis IA, De Francisci D, et al. Detailing the start-up and microalgal growth performance of a full-scale photobioreactor operated with bioindustrial wastewater. *Algal Res*. 2017;25:101–8. <https://doi.org/10.1016/j.algal.2017.04.030>.
- Ponnusamy S, Reddy HK, Muppaneni T, et al. Life cycle assessment of biodiesel production from algal bio-crude oils extracted under subcritical water conditions. *Biores Technol*. 2014;170:454–61. <https://doi.org/10.1016/j.biortech.2014.07.072>.
- Ragit SS, Mohapatra SK, Gill P, et al. Brown hemp methyl ester: transesterification process and evaluation of fuel properties. *Biomass Bioenergy*. 2012;41:14–20. <https://doi.org/10.1016/j.biombioe.2011.12.026>.
- Ragit SS, Mohapatra SK, Kundu K, et al. Optimization of neem methyl ester from transesterification process and fuel characterization as a diesel substitute. *Biomass Bioenergy*. 2011;35: 1138–44. <https://doi.org/10.1016/j.biombioe.2010.12.004>.
- Ross PJ. Taguchi techniques for quality engineering. New York: McGraw Hill Book Company; 1989. <https://doi.org/10.1002/qre.4680050312>.
- Sarin A, Arora R, Singh NP, et al. Effect of blends of Palm-Jatropha-Pongamia biodiesels on cloud point and pour point. *Energy*. 2009;34:2016–21. <https://doi.org/10.1016/j.energy.2009.08.017>.
- Seo YH, Han S, Han J. Economic biodiesel production using algal residue as substrate of lipid producing yeast *Cryptococcus curvatus*. *Renew Energy*. 2014;69:473–8. <https://doi.org/10.1016/j.renene.2014.03.062>.
- Shirveshan A, Almassi M, Ghobadian B, et al. Effects of biodiesel and engine load on some emission characteristics of a direct injection diesel engine. *Curr World Environ*. 2012;7:207–12.
- Shirveshan A. HC, CO, CO₂, and NO_x emission evaluation of a diesel engine fuelled with waste frying oil methyl ester. *Proc Soc Behav Sci*. 2013;75:292–7. <https://doi.org/10.12944/cwe.7.2.03>.
- Silva PT, Detmann E, Valadares Filho SC, et al. Evaluation of total and non-fatty ether extract in feeds and cattle faeces using two analytical methods. *Anim Feed Sci Technol*. 2011;163(2–4): 111–7. <https://doi.org/10.1016/j.anifeedsci.2010.10.012>.

- Smith VH, Sturm BSM, deNoyelles FJ, et al. The ecology of algal biodiesel production. *Trends Ecol Evol.* 2010;25:301–9.
- Sorguven E, Ozilgen M. Thermodynamic assessment of algal biodiesel utilization. *Renew Energy.* 2010;35:1956–66. <https://doi.org/10.1016/j.renene.2010.01.024>.
- Suganya T, Gandhi NN, Renganthan S. Production of algal biodiesel from marine microalga *Enteromorpha compressa* by two step process: optimization and kinetic study. *Biores Technol.* 2013; 128:392–400. <https://doi.org/10.1016/j.biortech.2012.10.068>.
- Taylor B, Xiao N, Sikorski J, et al. Techno-economic assessment of carbon-negative algal biodiesel for transport solutions. *Appl Energy.* 2013;106:262–74. <https://doi.org/10.1016/j.apenergy.2013.01.065>.
- Widjaja A, Chien CC, Ju YH. Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. *J Taiwan Inst Chem Eng.* 2009;40:13–20. <https://doi.org/10.1016/j.jtice.2008.07.007>.
- Woertz I, Feffer A, Lundquist T, et al. Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. *J Environ Eng.* 2009; ASCE 135:1115–22. [https://doi.org/10.1061/\(asce\)ee.1943-7870.0000129](https://doi.org/10.1061/(asce)ee.1943-7870.0000129).