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FULL LENGTH ARTICLE

# Kinetics of *Chlorella protothecoides* microalgal oil using base catalyst



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**KEYWORDS**

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 Methyl ester yields;  
 Reaction rate constant;  
 Activation energy

**Abstract** Due to continuous diminishing of fossil fuel resources and emission of greenhouse gases, the search for alternative fuels such as biodiesel and bioethanol has become inevitable. Biodiesel, also known as fatty acid methyl or ethyl ester, has emerged as a substitute for diesel because of similar fuel properties. Presently, biodiesel is produced from edible, non-edible and microalgal oil. *Chlorella protothecoides* (lipid content 14.6–57.8%) is being investigated as the potential microalgae species owing to high oil content, less land area required for cultivation and faster growth rate. The present investigation shows the results of the kinetics of transesterification of *C. protothecoides* microalgal oil carried out at optimum conditions of catalyst concentration, reaction temperature, molar ratio and reaction time. The percentage of methyl ester yield is the only parameter chosen to carry out the optimum parameter and the kinetics of transesterification. The reaction rate constant was to be  $0.0618 \text{ min}^{-1}$ . Furthermore, microalgal biodiesel is characterized for physico-chemical properties that are found to meet American (ASTM D6751) and Indian (IS 15607) standards, especially in cold flow properties and stability of conventional biodiesel.

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**1. Introduction**

Transport is the main sector consuming energy (almost 80% of total energy) and contributing to greenhouse gas (GHG) emissions resulting in global warming which is responsible for climate change [1]. Further, the depleting crude oil resources and difficulties in extraction and processing lead to the high cost of fuel [2]. This situation has initiated a search for alternative fuels like bioethanol and biodiesel that can be produced

from renewable biomass feedstocks [3,4]. Biodiesel, a mixture of mono-alkyl esters, is produced from oils or animal fats using a process termed as transesterification. Biodiesel from edible, non-edible and waste cooking oil is faced with serious problems of inadequacy of resources and would not be able to satisfy even a small fraction of the prevailing demands of transport fuels. Further, the high cost of biodiesel does not permit it to be used as a substitute for petro-diesel. Feedstock availability in enough quantities at cheaper price is therefore the key to success of biodiesel production and utilization on a large scale [5,6]. Recently, microalgae is viewed as the solution to the aforementioned problems owing to higher oil productivity (14.6–57.8% dry weight) within a short period of time (1–2 days) and also required less land area for cultivation

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**Table 1** Range of parameters selected.

Catalyst concentration (%) (w/v)	Temperature (°C)	Molar ration (methanol:oil) (v/v)	Time (min)
0.3–1.5	30–70	4–12:1	0–120

**Table 2** Properties of microalgal oil [32].

S. No.	Properties	Unit	Microalgal oil	Diesel
1	Density	kg l <sup>-1</sup>	0.910	0.836
2	Viscosity	cSt @ 40 °C	3.2	3.03
3	Flash point	°C	220	75
4	FFA	%	0.1	–
5	Acid value	%	0.2	< 0.005
6	Heating value	MJ/kg	32.73	40–45

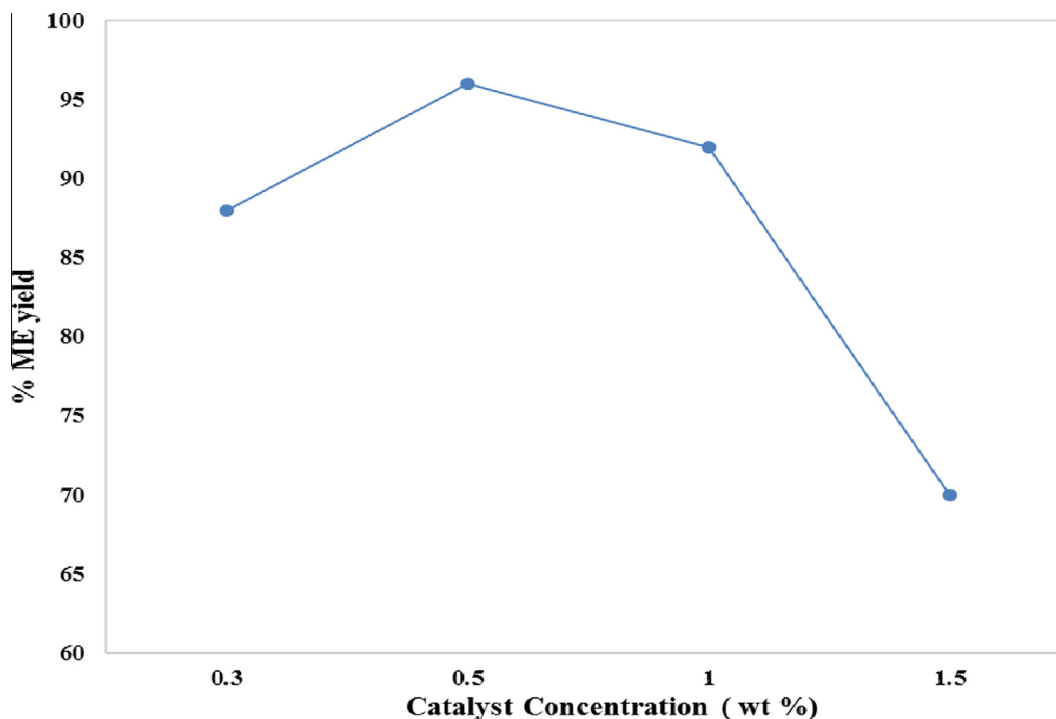
**Table 3** Optimum condition for ME production.

Reaction parameters	Transesterification
Catalyst & its concentration (%) (w/w)	NaOH (0.5)
Reaction temperature (°C)	60
Methanol to oil ratio (v/v)	7:1
Reaction time (min)	60
Methyl ester yield (%)	97.66

compared to top edible and non-edible oil crops [7]. Finally, microalgae is being viewed as a future source of biodiesel.

Several researchers have studied the kinetics of transesterification, to optimize the reaction parameters and to reduce the biodiesel production cost, which is still higher than the

diesel. The kinetics for catalytic, non-catalytic and supercritical transesterification are carried out by many authors. Darnoko et al., Freedman et al., Issariyakul et al., Zhang et al. reported the kinetics of palm oil using potassium hydroxide (KOH) as a catalyst and also investigated the activation energy in the range of 26.77–70.1 kJ/mol [8–11]. Yunus et al., Rathore et al., Ghosal et al., Aransiola et al., carried out kinetics of transesterification of non-edible oil using base catalyzed under the optimum condition of reaction parameters [12–15]. It is also described that the activation energy is in the range of edible oil. Nautiyal et al., Gutiérrez et al. studied the kinetics of microalgal and olive oil, respectively, using homogeneous acid catalyst [16,17]. It was examined that homogeneous acid catalyst necessitates less activation energy to start the chemical reaction than the homogeneous base catalyst. Jain et al., used both acid and base catalyst for the kinetics study of waste cooking oil and revealed that the base catalyst transesterification reaction is faster than the acid catalyst [18]. Deshmane et al., Birla et al., Kaur et al., Tran et al., Islam et al., carried out kinetics study using heterogeneous catalyst and reported that the transesterification reaction required a high molar ratio of > 15:1 and a high temperature > 60 °C for conversion than the homogeneous catalyst [19–23]. Diasakov et al., used non-catalyzed transesterification for soybean oil and concluded that the reaction required higher activation energy (117–128 MJ/mol) than the catalyzed transesterification [24]. Saka et al., Demirbas et al., Varma et al., Rathore et al., Chen et al., and Liu et al., reported the results of supercritical kinetics of edible, non-edible and microalgal oil [25–30]. Liu et al., studied the kinetics of microalgal oil using supercritical process and found that ME yield increased exceptionally at a molar ratio of 9:1 and 350 °C temperature. It was also illustrated that the activation energy required is less in the supercritical transesterification process due to a change in polarity of reactant

**Figure 1** % Effect of catalyst amount on percentage of methyl ester.

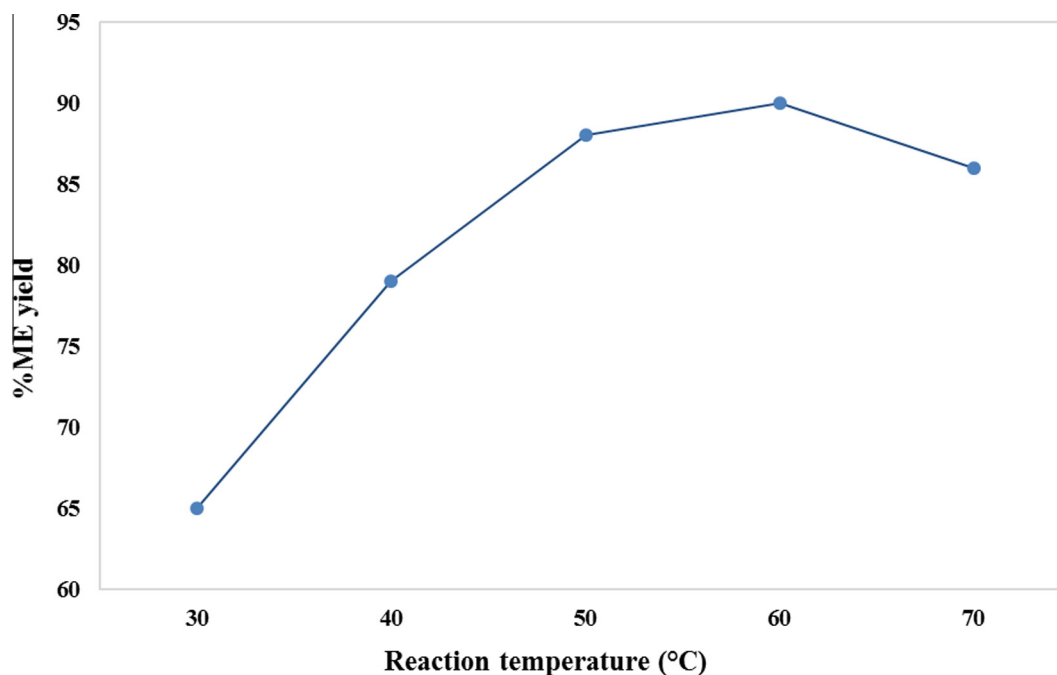


Figure 2 Variation in methyl ester yield (%) with temperature (°C).

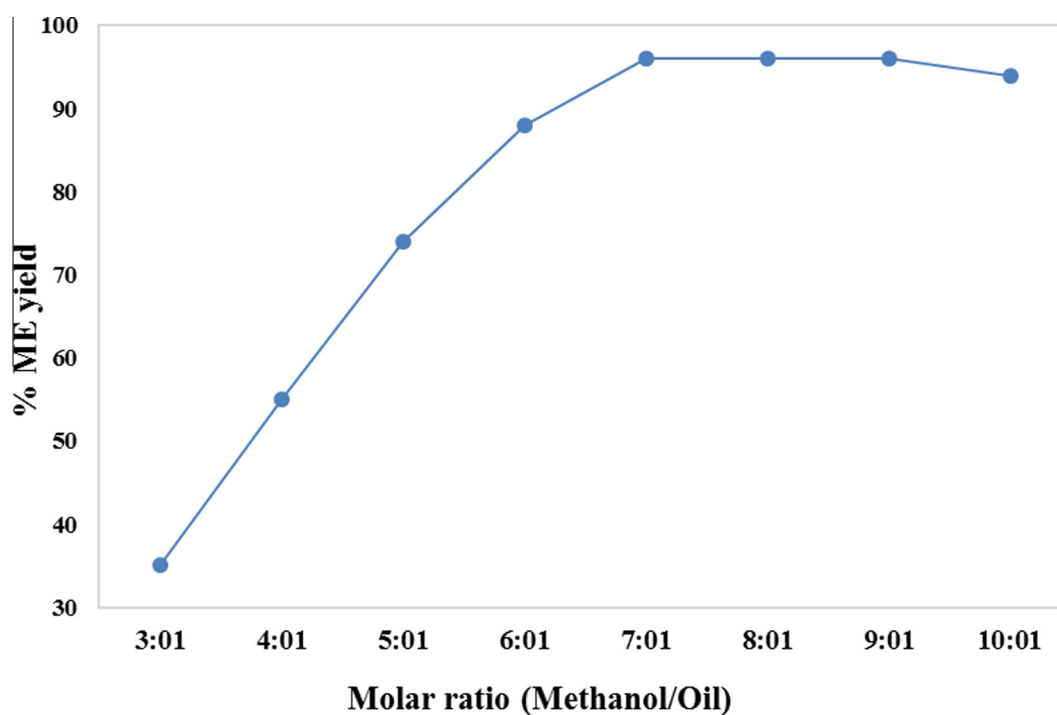


Figure 3 Variation in %ME formed with molar ratio (v/v).

[30]. This is the first study of its kind which deals with simple transesterification of *Chlorella protothecoides* microalgal species. Furthermore, sodium hydroxide (NaOH) is selected as catalyst due to its cheap price, less amount of catalyst amount required for complete conversion, high reactivity with oil etc. than the other base catalyst.

The above literature illustrates that extensive work is communicated on the kinetics study of edible and non-edible oil,

however, limited work is expressed in kinetics study of microalgal oil. The objective of this study is to find the optimum parameter of the transesterification reaction like catalyst conc., methanol to oil molar ratio, temperature and reaction time. The study also carried out the kinetics of microalgal oil using optimum parameters. Percentage methyl ester (%ME) is the only parameter chosen to study the optimum reaction parameters and kinetics of transesterification.

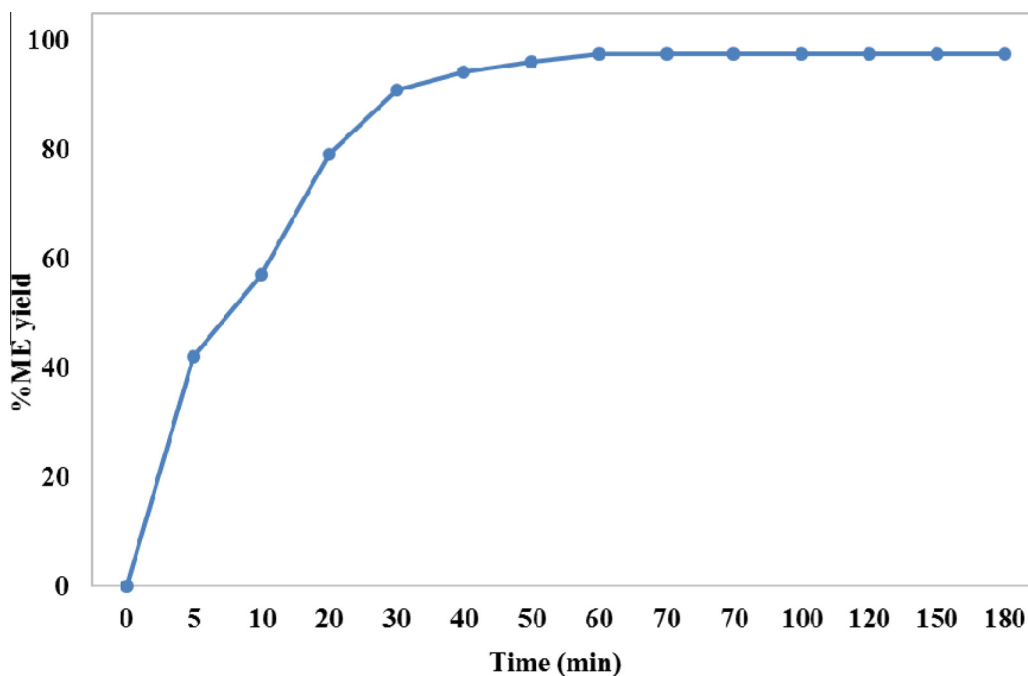


Figure 4 Variation in ME yield (%) with reaction time (min).

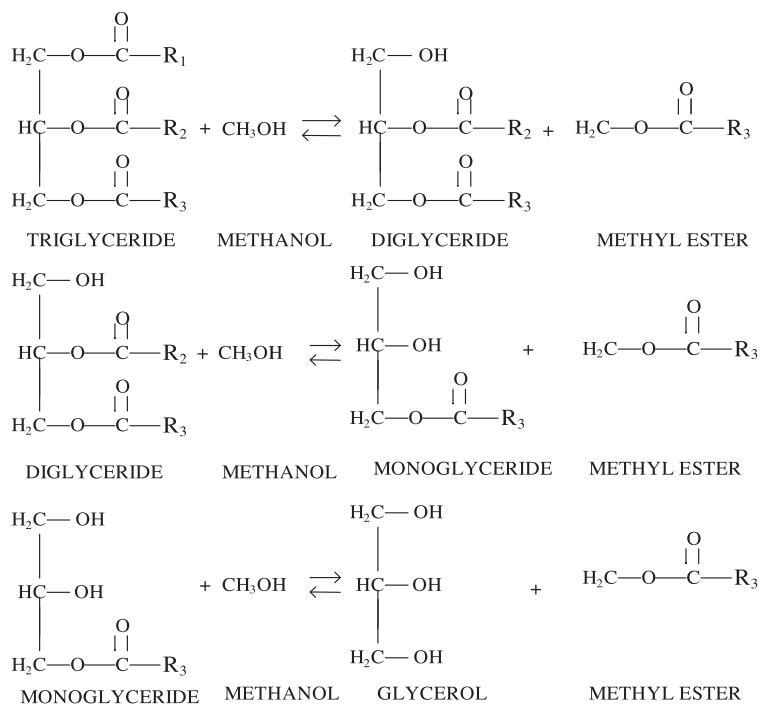


Figure 5 Scheme for step-wise transesterification reaction.

## 2. Experimental section

### 2.1. Materials

*C. protothecoides* microalgal oil was procured from M/s Soley Biotechnology Institute, Turkey. Other reactants including NaOH, methanol (CH<sub>3</sub>OH) and sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) were of analytical reagent (AR) grade and 99% pure. Microalgal oil

was filtered and then heated at 100 °C for 10 min to eliminate impurities and water particles.

### 2.2. Method: microalgal biodiesel production and its kinetics

Transesterification of microalgal oil was performed in a 500 ml round bottom flask equipped with a condenser, mechanical stirrer, thermometer and a sampling port. Oil was pre-heated

**Table 4** Rate constant of transesterification of *Chlorella protothecoides* microalgal oil.

Catalyst	Rate constant $K$ , [min <sup>-1</sup> ]	Residual norm ( $S$ )
NaOH	0.0618	0.04609

to remove water particles before being injected into the reactor. Calculated amounts of CH<sub>3</sub>OH and NaOH were dissolved separately and then charged into the reactor while stirring started immediately. The biodiesel samples were taken out from the reactor at a predetermined time and estimated the %ME yield. After 2 h, two different layers of ME yield and glycerol were formed. Both layers of ME and glycerol were separated by using a separating funnel. The ME yield was washed using water, heated to eliminate moisture and dried over Na<sub>2</sub>SO<sub>4</sub>. From extensive literature review, the range of experiment was chosen and shown in Table 1.

After optimizing reaction parameters, the kinetics of microalgal oil was studied under these reaction parameters. The stirring rate is fixed at 100 rpm [31]. The reaction rate constant and activation energy of conversion reaction are calculated on the basis of ME yield.

### 2.3. Analysis of ME yield

The reaction samples were taken from the reactor at a predetermined time and analyzed by gas chromatography (Netal make) using a capillary column and a flame ionization detector. Nitrogen gas was used as carrier gas at a defined flow rate. Temperature of the oven was maintained at 250 °C and analytical time was fixed at 30 min. The %ME was analyzed using standards EN 14103:2003 [18].

## 3. Results and discussion

The optimum reaction conditions and kinetics of microalgal oil have been investigated and discussed in the following sections.

### 3.1. Physico-chemical properties of microalgal oil

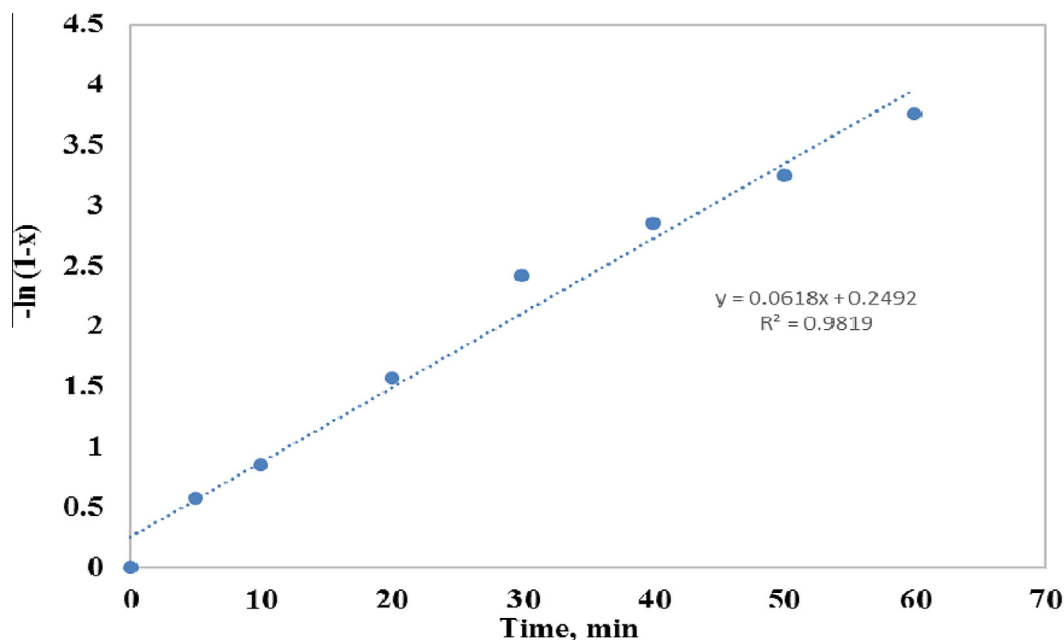
The physicochemical properties of microalgal oil were measured using the ASTM method and reported in Table 2. Microalgal oil were characterized for fuel properties like density, viscosity, flash point, heating value and also chemical properties like free fatty acid and acid value. Both fuel and chemical properties predict the nature of fuel. In this study, microalgal oil has less FFA content which can ensure high conversion yield than the high FFA content in oil.

The above table shows that the viscosity of *C. protothecoides* microalgal oil is found as 3.2 cSt at 40 °C which is in the range of diesel (3.03 cSt). This indicates that microalgal oil does not have any flow problem in the engine and performs a similar operation to diesel. However, microalgal oil has a high molecular weight of 850, which is the reason for soot formation and carbon deposit inside the engine [33]. In order to eliminate such kinds of problems, the microalgal oil is converted into biodiesel by transesterification. Furthermore, the fuel and chemical properties of microalgal oil is also in the range of diesel.

### 3.2. Determination of optimum reaction parameters

#### 3.2.1. Catalyst concentration

The impact of catalyst concentration on ME yield was examined and depicted in Fig. 1. The molar ratio of 8:1, 50 °C temperature and reaction time of 120 min were fixed. ME yield was found to be minimum at a concentration of 0.3% (w/w)

**Figure 6** Integral form of rate constant versus reaction time.

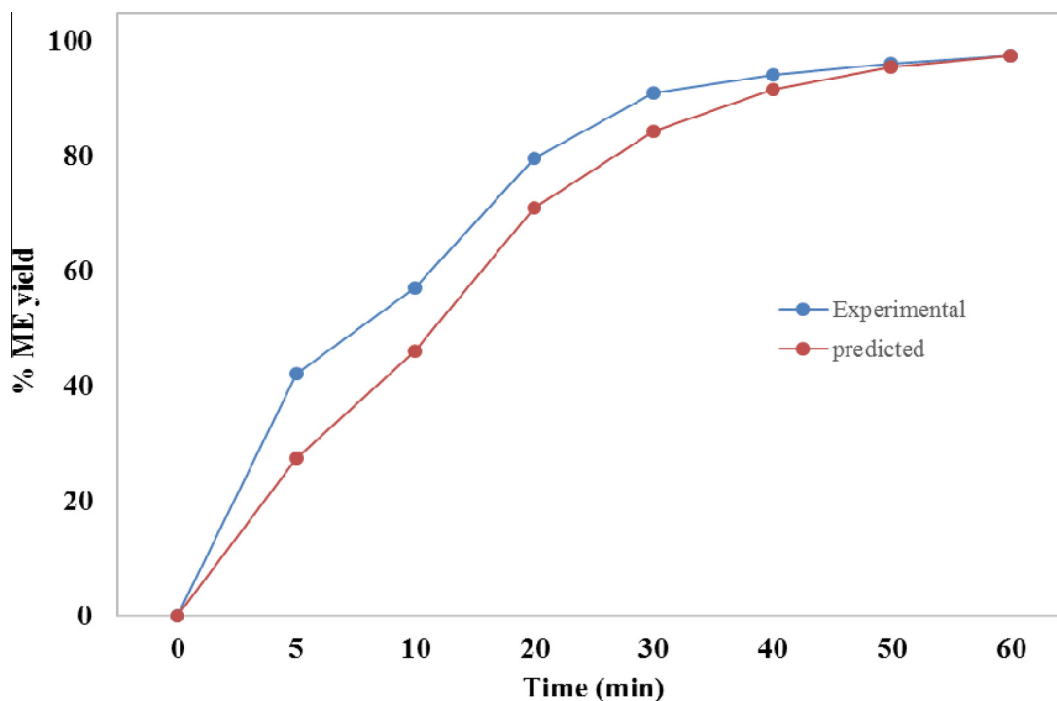


Figure 7 Comparison of experimentally determined and calculated methyl ester.

because the conversion of oil into ME yield is not complete at low concentration. The ME yield started increasing with an increase in catalyst concentration and reached a maximum value at 0.5% concentration, however, with further increase in catalyst concentration, the ME yield start decreasing. It is due to the fact that at excess concentration of catalyst the separation of ME yield from glycerol becomes difficult resulting in lower ME yield. It is concluded that maximum ME yield is obtained at 0.5% (w/w) catalyst concentration.

### 3.2.2. Reaction temperature

The variation in ME yield w.r.t temperature was investigated as shown in Fig. 2 under the optimum conditions of catalyst concentration 0.5% (w/w) and molar ratio of 8:1. The ME yield obtained was minimum at 30 °C and maximum at 60 °C, this is due to the fact that the conversion of oil into ME yield is faster at high temperature compared to low temperature. However, with a further increase in temperature, the ME yield started decreasing because CH<sub>3</sub>OH started boiling (boiling temperature of methanol is 64.7 °C). Thus, the optimum temperature was found to be 60 °C.

### 3.2.3. Molar ratio

The molar ratio of CH<sub>3</sub>OH to oil is one of the most important factors that can affect the ME yield owing to the fact that higher concentration of CH<sub>3</sub>OH increases the ME yield. Transesterification process requires a minimum amount of 3:1 M ratio to yield 3 mol of ester and 1 mol of glycerol, but excess alcohol was required to drive the reaction close to completion. Therefore, the effect of methanol was investigated, in the range of 3:1–12:1 (v/v) and depicted in Fig. 3. ME yield was increased from 35% to 96% with a rise in molar ratio from 3:1 to 7:1 at optimum conditions of NaOH concentration

of 0.5%, temperature 60 °C, however, a further increase in molar ratio did not affect the ME yield. Hence, the maximum ME yield was obtained at 7:1 M ratio.

### 3.2.4. Reaction time

To obtain the optimum reaction time, the conversion of microalgal oil was investigated under the aforementioned optimized reaction condition of catalyst concentration of 0.5% (w/w), reaction temperature of 60 °C and CH<sub>3</sub>OH to oil molar ratio of 7:1 as represented in Fig. 4. The reaction time is varied from 0 to 180 min. the ME yield started increasing from 0 min and becomes maximum at 60 min. Further increase in reaction time did not affect the ME yield because the conversion of oil into ME yield was completed. Thus, the maximum ME yield was obtained in 60 min.

On the basis of results obtained, the optimum conditions for ME production from microalgal *C. protothecoides* are compiled in Table 3.

The above table concludes that 97.66% ME yield was obtained under the reaction conditions of catalyst conc. of 0.5% (w/w), methanol to oil molar ratio of 7:1, reaction temperature of 60 °C and reaction time of 60 min.

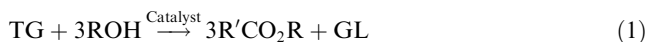
### 3.3. Kinetics study of transesterification of *Chlorella protothecoides* microalgal oil

The formation of ME yield has been investigated under the optimized conditions (catalyst conc. of 0.5%, methanol to oil molar ratio of 7:1). Transesterification reaction proceeded in three steps as given below [34] (see Fig. 5):

However, the rate of conversion for three steps is difficult in terms of operation, cost and time. Therefore, 3 step conversion has been simplified and comprised into one step as given in Eq. (1).

**Table 5** Physicochemical properties of *Chlorella protothecoides* biodiesel as per standard methods [13].

Properties (unit)	ASTM 6751	ASTM 6751 limit	IS 15607	Biodiesel specification IS 15607	Microalgal biodiesel
Density (gm/cm <sup>3</sup> )	ASTM D4052	–	IS1448	0.86–0.90	0.860
Viscosity (cSt @ 40 °C)	ASTM D445	1.9–6.0	IS1448	3.5–5.0	2.8
Flash point (°C)	ASTM D93	130 min	IS1448	120 min	124
CP (°C)	ASTM D2500-11	–	IS1448	–	–8
PP (°C)	ASTM D97-12	–	IS1448	–	–11
Oxidation stability (h)	EN 14112	3	EN 14112	6 h, min	11.8



Several authors used %ME yield as the only parameter to monitor kinetics of transesterification and intermediate products like DG, MG have been ignored [18,24,25,31]. The rate constant of the reaction can be determined on the basis of increased amount of ME yield that occurs in some reaction time interval as given below [31,34].

For an irreversible reaction the rate is defined as:

$$-\text{rate} = kC_{\text{ME}} \quad (2)$$

where,  $C_{\text{ME}}$  is the methyl ester concentration and  $k$  is the rate constant for the first order irreversible kinetic model. Since the ME concentration is related to the conversion degree of ME,  $x$ , by the following equation:

$$C_{\text{ME}} = C_{\text{ME}0}(1 - x) \quad (3)$$

Eq. (2) can be transformed into:

$$\frac{dx}{dt} = k(1 - x) \quad (4)$$

After integration the Eq. (4) changes into:

$$-\ln(1 - x) = kt \quad (5)$$

where  $\text{ME}_0$ : methyl ester at time  $t = 0$ .

Using Eq. (5), the reaction rate constant under optimum conditions of temperature, catalyst concentration and molar ratio has been computed as  $0.0618 \text{ min}^{-1}$  as shown in Fig. 6. The obtained rate constant is comparable to the values reported in the literature. The values of rate constant is similar to the values described for base catalyzed methanolysis of mahua oil (0.08) [35], smaller than the value of the rate constant determined by methanolysis of jatropha oil at  $45^\circ\text{C}$  using KOH (1.26) [35], but higher than the rate constant reported by Jain et al. [31] which was  $0.008 \text{ min}^{-1}$ .

The predicted rate constant was estimated and compared with the experimentally obtained data using the least-square nonlinear optimization function in Matlab (Fig. 7).

#### 4. Accuracy of parameter estimates

A measure of the accuracy of parameter estimates was obtained through the quantity residual norm  $S$  defined as:

$$S = \sum_i ([\text{ME}]^{\text{exp}} - [\text{ME}]^{\text{pred}})^2$$

where the index  $i$  is run over all experiment points. The values  $S$  are given in Table 4.

#### 5. Biodiesel characterization

The microalgal biodiesel is characterized as per the standard methods and is depicted in Table 5. It is concluded from the table that the fuel properties of microalgal biodiesel meets the specification of both ASTM and Indian standards ISO 15607. The microalgal biodiesel has a low viscosity in the range of conventional biodiesel which may provide smooth engine operation and also increase the engine life. The flash point of microalgal biodiesel is high which provides safer transportation. Furthermore, high oxidation stability indicates that biodiesel can be stored for a long duration without deteriorating its properties and also having good cold flow properties, provide smooth working in cold weather conditions.

#### 6. Conclusion

The results of the present study shows that the highest biodiesel yield (97.66%) was obtained at 0.5% catalyst conc. (w/w), methanol to oil molar ratio of 7:1, reaction temperature of  $60^\circ\text{C}$  and reaction time of 60 min. The kinetics of *C. protothecoides* microalgal oil was studied w.r.t. %ME yield by considering that the transesterification process is a first order irreversible reaction. Kinetics data such as reaction rate, temperature and %ME yield will be used for the production process development and the reactor design, operation, scale-up and also to predict the extent of the reaction at any time under particular conditions. Therefore, kinetic data are of crucial importance for the design for process development. Furthermore, the microalgal biodiesel fuel properties meet ASTM and IS standards. Microalgal biodiesel has high oxidation stability and better cold flow properties. The results favor the possibility of using microalgal biodiesel in IC engines as a substitute for diesel.

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#### References

- [1] G. Vicente, M. Martinez, J. Aracil, *Bioresour. Technol.* 99 (2008) 9009–9012.
- [2] < <https://www.academia.edu/2575793/18> > .
- [3] A.L. Ahmad, N.H. Mat Yasin, C.J.C. Derek, J.K. Lim, *Renewable Sustainable Energy Rev.* 15 (2011) 584–593.
- [4] T.M. Mata, A.A. Martins, N.S. Caetano, *Renewable Sustainable Energy Rev.* 14 (2010) 217–232.

- [5] A.K. Khan, H.M.Z. Rashmi, S. Prasad, U.C. Banerjee, *Renewable Sustainable Energy Rev.* 13 (2009) 2361–2372.
- [6] J. Janaun, N. Ellis, *Renewable Sustainable Energy Rev.* 14 (2009) 1312–1320.
- [7] L. Brennan, P. Owende, *Renewable Sustainable Energy Rev.* 14 (2010) 557–577.
- [8] D. Darnoko, M. Cheryan, *J. Am. Oil Chem. Soc.* 77 (2000) 1263–1267.
- [9] B. Freedman, R.O. Butterfield, E.H. Pryde, *J. Am. Oil Chem. Soc.* 63 (1986) 1375–1380.
- [10] T. Issariyakul, A.K. Dalai, *Can. J. Chem. Eng.* 90 (2012) 342–350.
- [11] L. Zhang, B. Sheng, Z. Xin, Q. Liu, S. Sun, *Bioresour. Technol.* 101 (2010) 8144–8150.
- [12] R. Yunus, A.M. Syam, *Int. J. Sustainable Energy* 30 (2011) 175–183.
- [13] V. Rathore, S. Tyagi, B. Newalkar, R.P. Badoni, *Fuel* 140 (2015) 597–608.
- [14] D. Ghosal, R.R. Pradhan, *Int. J. Emerg. Technol. Adv. Eng.* 4 (2014) 310–314.
- [15] E.F. Aransiola, M.O. Daramola, T.V. Ojumu, B.O. Solomon, S. K. Layokun, *Sci. Res.* 2 (2013) 83–89.
- [16] P. Nautiyal, K.A. Subramanian, M.G. Dastidar, *Fuel* 135 (2014) 228–234.
- [17] M.C. Gutiérrez, J.A. Siles, A.F. Chica, M.A. Martín, *Biomass Bioenergy* 62 (2014) 93–99.
- [18] S. Jain, M.P. Sharma, S. Rajvanshi, *Fuel Process. Technol.* 92 (2011) 32–38.
- [19] V.G. Deshmane, Y.G. Adewuyi, *Fuel* 107 (2012) 474–482.
- [20] A. Birla, B. Singh, S.N. Upadhyay, Y.C. Sharma, *Bioresour. Technol.* 106 (2012) 95–100.
- [21] N. Kaur, A. Ali, *Fuel Process. Technol.* 119 (2014) 173–184.
- [22] D.T. Tran, Y.J. Lin, C.L. Chen, J.S. Chang, *Bioresour. Technol.* 145 (2013) 193–203.
- [23] M.R. Islam, Y.M. Kurlle, J.L. Gossage, T.J. Benson, *Energy Fuels* 27 (2013) 1564–1569.
- [24] M. Diasakov, A. Louloudi, N. Papayannakos, *Fuel* 77 (1998) 1297–1302.
- [25] S. Saka, D. Kusdiana, *Fuel* 80 (2001) 693–698.
- [26] A. Demirbas, *Energy Convers. Manage.* 43 (2002) 2349–2356.
- [27] M.N. Varma, G. Madras, *Ind. Eng. Chem. Res.* 46 (2007) 1–6.
- [28] V. Rathore, G. Madras, *Fuel* 86 (2007) 2650–2659.
- [29] J. Chen, H.U. Xianguo, X. Wang, X.U. Yufu, H.U. Enzhu, *Adv. Mater. Res.* 578 (2012) 73–77.
- [30] <[http://surface.syr.edu/bce\\_thesis/3/](http://surface.syr.edu/bce_thesis/3/)>.
- [31] S. Jain, M.P. Sharma, *Bioresour. Technol.* 101 (2010) 7701–7706.
- [32] M. Kumar, M.P. Sharma, *J. Mater. Environ. Sci.* 2 (2014) 757–766.
- [33] Q. Feng, A. Jalali, A.M. Fincham, *Combust. Flame* 159 (2012) 1876–1893.
- [34] I. Lukić, Ž. Kesić, D. Skala, *Chem. Eng. Technol.* 37 (2014) 1879–1884.
- [35] G.R. Kumar, R. Ravi, A. Chadha, *Energy Fuels* (2011).