CI&CEQ
Chemical Industry & Chemical Engineering Quarterly

Available on line at
Association of the Chemical Engineers of Serbia *AChE*
www.ache.org.rs/CICEQ

Chem. Ind. Chem. Eng. Q. 23 (3) 367–375 (2017) CI&CEQ

DIDEM ÖZÇIMEN
M. ÖMER GÜLYURT
BENAN İNAN

Department of Bioengineering, Faculty of Chemical and Metallurgical Engineering, Yıldız Technical University, Davutpasa Campus, Istanbul, Turkey

SCIENTIFIC PAPER

UDC 662.756.3:582.26:66.095.13:54

OPTIMIZATION OF BIODIESEL PRODUCTION FROM *Chlorella protothecoides* OIL *VIA* ULTRASOUND ASSISTED TRANSESTERIFICATION

Article Highlights

- Ultrasound-assisted transesterification of *C. protothecoides* oil was proposed
- The optimum conditions were determined using factorial design method
- The catalyst/oil ratio was found as the most effective parameter on methyl ester yield
- This method is an alternative way to produce biodiesel efficiently and cost effective
- The highest FAME was obtained with 40 min, 9:1 methanol/oil mole ratio and 1.5% catalyst in oil

Abstract

There is a growing interest in biodiesel as an alternative fuel for diesel engines because of the high oil prices and environmental issues related to massive greenhouse gas emissions. Nowadays, microalgal biomass has become a promising biodiesel feedstock. However, traditional biodiesel production from microalgae consumes a lot of energy and solvents. It is necessary to use an alternative method that can reduce the energy and alcohol consumption and save time. In this study, biodiesel production from Chlorella protothecoides oil by ultrasound assisted transesterification was conducted and effects of reaction parameters such as methanol:oil ratio, catalyst/oil ratio and reaction time on fatty acid methyl ester yields were investigated. The transesterification reactions were carried out by using methanol as alcohol and potassium hydroxide as the catalyst. The highest methyl ester production was obtained under the conditions of 9:1 methanol/oil mole ratio, 1.5% potassium hydroxide catalyst in oil, and for reaction time of 40 min. It was also found that catalyst/oil molar ratio was the most effective parameter on methyl ester yield according to statistical data. The results showed that ultrasound-assisted transesterification may be an alternative and cost effective way to produce biodiesel efficiently.

Keywords: algal biodiesel, Chlorella protothecoides, methyl ester, transesterification, ultrasound.

Today, substitution of non-conventional sources such as biodiesel over traditional ones is inevitable due to the depletion of petroleum based sources, high oil prices, and air pollution caused by the combustion of conventional petroleum-based diesel. Development of industrial growth, transportation, and many other basic human needs are met with petroleum based fuels. Worldwide average demand of oil and liquid fuels is nearly 96 million barrels of per day according to the agencies and oil companies for 2016 [1-3]. In addition to this, emissions of many greenhouse gases (GHG) and pollutants such as particulate matter (PM), carbon monoxide (CO), carbon dioxide (CO_2), nitrogen oxides (NO_x) and unburnt hydrocarbon (UBHC) have significant effects on global warming and human health. Biodiesel is defined as a renewable fuel for diesel, which can be produced from various feedstocks such as vegetable oils, waste oils and microbial oils. Studies show that using biodiesel improves

Correspondence: D. Özçimen, Department of Bioengineering, Faculty of Chemical and Metallurgical Engineering, Yıldız Technical University, Davutpasa Campus, 34220, Istanbul, Turkey.
E-mail: didem.ozcimen@gmail.com
Paper received: 6 March, 2016
Paper revised: 26 September, 2016
Paper accepted: 12 October, 2016

https://doi.org/10.2298/CICEQ160306051O

the combustion in the engine due to its high cetane number and oxygen content. This efficient combustion also significantly reduces the emissions of PM, HC, and CO [1]. As mentioned before, many feedstocks can be utilized for biodiesel production. As the first generation biodiesel feedstock, vegetable oil is the most common raw material for biodiesel production. However, cultivation of vegetable plants is also contributing to GHG emissions because of the fertilizer used for their growth. Besides that, there is a debate on cultivating them for food or use in biodiesel production. Storage of these feedstocks is another issue due to decomposition over time. To overcome these problems, waste oils are considered to be utilized in biodiesel production instead of vegetable oils. However, there is a purification requirement for waste oil, due to different waste particles in the oil can affect the combustion and reduce the performance of engine. Microalgae have come into prominence in the last decade due to the productivity of microalgae and being an alternative for vegetable feedstock. It is known that there are more than 30,000 algal species which have been defined [4]. As photosynthetic microorganisms, they can perform photosynthesis by utilizing sunlight and carbon dioxide in the atmosphere. In addition to having 30% lipid content in microalgae cells, lipid production of microalgae is about 4.5-7.5 metric t ha^{-1} $year^{-1}$ without optimizing the growth conditions. When these data compared with other feedstocks, lipid production of microalgae is higher than the production from other sources such as soybeans (0.4 metric t ha^{-1} $year^{-1}$), palm oil (3.62 metric t ha^{-1} $year^{-1}$), and jatropha (4.14 metric t ha^{-1} $year^{-1}$) [5]. Microalgae produce different amounts of fatty acids with different compositions. These fatty acids contain medium-chain (C10–C14), long chain (C16–C18) and very long chain (>C20) fatty acids and their derivatives. However, most microalgae species accumulate neutral lipids in the form of triacylglycerol under stress conditions by changing their biosynthetic pathways. Microalgal fatty acids range from 12 to 22 carbons in length and can be either saturated or unsaturated. Fatty acid composition of microalgal oil basically consists of mixture of unsaturated fatty acids. This mixture includes palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2) and linolenic acid (C18:3). In addition to these fatty acids, saturated fatty acids, palmitic and stearic acid are available in a small proportion [6]. The number of double bonds in the fatty acid chains never exceeds 6 and almost all of the unsaturated fatty acids are cis isomers [7]. The quality of microalgal biodiesel meets American Society for Testing and Materials (ASTM) Biodiesel Standard D6751, thus can substitute for petroleum diesel [8]. Fuel properties such as oxidation stability and cold filter plugging point are affected from fatty acid composition of the oil. Methyl ester of oleic acid provides a better balance between these properties. Conversely, linoleic acid has very high oxidation instability, which means it can be easily oxidized. For this reason, microalgae with high oleic acid and low linoleic acid content are suitable for high quality biodiesel production [9]. Microalgal lipid content varies from species in a range of 5 to 77 wt.% dry biomass according to cultivation conditions and genetic engineering applications. Microalgae accumulate lipid under nitrogen limitation, yet excess carbon is assimilated and converted to TAG by microalgal cells when lipid synthesis is carried out in phase of balance cell growth. Important parameters such as nutrient starvation, salinity, pH, temperature and light intensity can change the fatty acid composition by altering the unsaturation and formation of polar lipids. In this study, *Chlorella protothecoides* oil was evaluated for biodiesel production. *Chlorella protothecoides* is a green microalgae species belonged to *Chlorella* genus. *Chlorella protothecoides* is presented as promising microalgal feedstock due to its capability to achieve a relative high biomass and lipid accumulation using different carbon sources under various environmental conditions [10]. Microalgae are currently not economically viable because of the expensive operation and capital costs in the process steps such as algae growth, harvesting, dewatering, and conversion to a fuel. For fuel production, transesterification of oil is the most common technique applied in today. However, high amount of energy input, solvent usage and undesired by-product formation lead the researchers to evaluate novel methods in biodiesel production. As a novel method, ultrasound assisted process meets green chemistry principals and has a wide application area in chemical processes including biodiesel production. Mixing is a significant parameter that affects biodiesel yield in transesterification reactions. Smaller droplets are created by powerful mixing, which increases the contact areas between the oil phases. The required activation energy is also provided by this effective mixing for initiating transesterification reactions. Ultrasonication is an effective mixing method that provides needed activation energy and better liquid-liquid mass transfer [11,12]. Ultrasonic baths and ultrasonic probes are usually operated at a fixed frequency. Ultrasound assisted transesterification of oil has significant advantages compared to conventional stirring methods such as: reduced reaction time, higher rate of chemical reaction, and lower mole

ratio of alcohol requirement, increasing the yield and conversion. Production of biodiesel *via* ultrasound is an effective and economically functional method due to its time and energy savings [13]. The effect of ultrasound is clearly explained by Gryglewicz who found that the transesterification reaction rate can be enhanced by ultrasound as well as by introducing an appropriate reagent into a reactor to promote methanol solubility in the rapeseed oil. In this study, calcium compounds were examined to produce methyl esters of rapeseed oil. It was reported that the transesterification of rapeseed oil by methyl alcohol can be catalysed effectively by basic alkaline-earth metal compounds: calcium oxide, calcium methoxide and barium hydroxide. It is known that, ultrasonic waves resulted an increase of reaction rate if the catalyst is soluble or well dispersed in the liquid phase of the reagents. In the considered case, sodium and barium hydroxides are well soluble in solvents such as methanol [14]. Stavarache *et al.* also indicated that the reaction time is much shorter (10–40 min) than for mechanical stirring, the quantity of required catalyst is 2 or 3 times lower, and the alcohol/oil mole ratio used is only 6:1 *via* ultrasound process [15].

As it was mentioned before, due to algal biofuel production is expensive, in this respect, cost effective methods such as ultrasound assisted biodiesel production should be considered. Although there are lots of studies about ultrasound assisted biodiesel production from various vegetable or waste oils, there is a just few studies on microalgal biodiesel production via ultrasound assisted process. In the study of Ehimen *et al.*, in situ transesterification of microalgae by ultrasound technique was carried out in 1 h with the use of methanol/oil ratio of 315:1. At the end of the reaction, it was found that 0.295±0.003 g biodiesel/g dry *Chlorella* was obtained and it was showed that this was higher than mechanically stirred in situ technique [16]. Most of the studies that used algae as feedstock for biodiesel production *via* ultrasound assisted process were carried out with in-situ transesterification. Even though there are studies on biodiesel production from algal sources, to our knowledge, there have been no investigations of ultrasound assisted biodiesel production from *C. prototheco-ides* and the transesterification parameters on biodiesel yield [17-21]. The aims of this work are to conduct transesterification of *C. prototheco-ides* oil by using ultrasound assisted method and observe the effect of reaction parameters that affect the purity of the ester product. In addition, parameters which include the mole ratio of methanol and microalgal oil, alkaline catalyst/oil ratio, and the reaction time were also analyzed statistically by using factorial design technique to provide accuracy and reproducibility for further studies. Determination of methyl ester properties of algal product was also performed.

EXPERIMENTAL

Materials

Methanol (99.5%), and potassium hydroxide were purchased from Merck (Darmstadt, Germany). All the chemicals used for transesterification experiments and characterization tests were carried out with these analytical reagent grade chemicals. As raw material, *C. prototheco-ides* oil was obtained from Soley Biotechnology Institute (CA, USA). Characteristics of *C.prototheco-ides* oil are presented in the Table 1 [22].

Table 1. Properties of C. prototheco-ides oil

Property	Unit	Result
Density at 15 °C	kg/m³	867
Viscosity at 40 °C	mm²/s	3.8
Flash point	°C	124
Carbon residue (on 10% distillation residue)	mass%	0.2
Total contamination	mg/kg	2
Oxidation stability, 110 °C	h	12
Calorific value	MJ/kg	37.49
Acid value	mg KOH/g	0.3
Iodine value	mg KOH/g	47
Water content	mg/kg	80
Sulfur content	mg/kg	2
Phosphorus content	mg/kg	3
Monoglyceride content	mass%	0.31
Diglyceride content	mass%	1.22
Triglyceride content	mass%	97.26

Experimental procedure

Transesterification reactions were carried out under the conditions of different methanol:microalgal oil mole ratios (3:1, 6:1 and 9:1) in the presence of varying KOH catalyst in oil content (0.5, 1 and 1.5 wt.%) at different reaction times (20, 30 and 40 min). A Bandelin Sonopuls ultrasonic probe (20 kHz frequency and 200 W power) was used to conduct transesterification reactions, which were carried out in a 250 mL round bottom flask at autogenous temperature (60-65 °C). An ice bath was used to cool the product that composed of methyl ester-glycerine mixture obtained at the end of the reaction. After the cooling, centrifugation was performed at 5000 rpm for 5 min and created two phases as bottom and super-

natant. Glycerin, soap, methanol and impurities were separated from the product as bottom phase and methyl ester product was separated as supernatant. The obtained methyl ester was analyzed by gas chromatography to determine its fatty acid methyl ester (FAME) yield and profile. Experiments were repeated two times and the results obtained from each experiment were the average of these data.

Analysis of FAME content

Analyzing fatty acid methyl ester (FAME) and the determination of FAME content were carried out with FID gas chromatography (YL Instruments 6100 GC). ZB-FFAP (30 m×0.32 mm×0.25 μm) column was used for GC analysis. Analyses were carried out according to our previous study. The temperature program of the column was started with the temperature of 75 °C, and heated to 140 °C with heating rate of 16 °C/min, and then it was heated to 300 °C with heating rate of 15 °C/min, respectively. Hydrogen gas was used as carrier gas and gas flow was adjusted to 2 ml/min. The injection of the samples volume was 1 μl. In order to identify the peaks, methyl margarate (C17:0) was used as an internal standard and the samples were prepared with mixing methyl margarate and *n*-heptane for GC analysis. Peak identification of fatty acids was carried out according to comparison of the retention times [22].

RESULTS AND DISCUSSION

Statistical evaluation of the experimental results

In this study, 2^3 factorial design was used to provide information regarding the interior of the experiment region and it was utilized to observe the effects and interactions of the amounts of methanol and KOH, and reaction time on the yield of FAME. In Table 2, experimental results of transesterification of *C. protothecoides* oil are presented. The experimental variables consisting of methanol/oil mole ratio (X_1), catalyst/oil ratio (X_2), reaction time (X_3) and their levels were given as:

X_1: 9:1 (superior level), 6:1 (central level), 3:1 (inferior level);

X_2: 1.5 (superior level), 1 (central level), 0.5% (inferior level);

X_3: 20 (superior level), 30 (central level), 40 min (inferior level).

X_1, X_2 and X_3 are the normalized values of the experimental variables in the regression equation. Regression equation was obtained to evaluate the methyl ester yield and to understand the effects of experimental variables on the yield. The design matrix was examined statistically to define and measure the main effects quantitatively by using the analysis of variance (ANOVA) technique. For an approximate calculation of the methyl ester yield values of microalgal oil with this statistical analysis, regression Eq. (1) was developed:

$$y_{(\text{FAME }(\%))} = 0.709 + 0.0427X_1 + 0.134X_2 + 0.0705X_3 \quad (1)$$

The coefficient of determination for regression Eq. (1) was found as 0.95.

According to the regression equation, it was found that the coefficient of catalyst/oil molar ratio was the highest among all the variables, and therefore effect of catalyst/oil mole ratio on the methyl ester yield is the strongest. The reaction time and the methanol/oil ratio also affected the methyl ester yield, positively. The interactive effect of X_1X_2 (methanol/oil mole ratio and catalyst/oil ratio), X_2X_3 (catalyst/oil ratio and time) and X_1X_3 (methanol/oil mole ratio and time) has very small coefficient values, this interactional effects were not presented in equation of regression model.

The ANOVA method compares the variances within and between the groups. The reliability of the model is confirmed with this technique. ANOVA parameters for the model equation are presented in Table 3. In order to observe these parameters, Mini-

Table 2. The methyl ester yields and design matrix obtained at different reaction conditions

Experiment No.	Methanol:oil mole ratio	Catalyst to oil share, wt.%	Time, min	X_1	X_2	X_3	Total area (uV.Min)	Total area, %
1	3:1	0.5	20	-1	-1	-1	217.41	47.34
2			40	-1	-1	1	265.82	57.88
3		1.5	20	-1	1	-1	349.30	76.06
4			40	-1	1	1	381.23	83.01
5	9:1	0.5	20	1	-1	-1	246.02	53.57
6			40	1	-1	1	317.36	69.10
7		1.5	20	1	1	-1	349.73	76.15
8			40	1	1	1	457.26	99.56
9	6:1	1	30	0	0	0	345.05	75.13

tab statistical software (Minitab 14) was used. The *F*-value of 34.14 with a very low probability value ($p < 0.01\%$) implies that the model is significant. Moreover, a high value of correlation coefficient shows a close agreement between predicted value and actual value of response. The determination coefficient (R^2) controls the suitability of the model. As the value of R approaches 1, it demonstrates better correlation between observed and predicted values [23]. The determination coefficient value was determined as $R^2 = 0.95$ and it represented that only about 5% of the total variation was not explained by the respective model. Besides that, the value of the adjusted determination coefficient was found very high as adj. $R^2 = 0.928$. When the normalized values (−1, 0, 1) of experimental variables were converted to real values, the real regression Eq. (2) was obtained. The equation developed with real values helps determining methyl ester yield value without doing any experiments in the investigated parameter range:

$$y_{real} = 14.5 + 1.42X_1 + 26.7X_2 + 0.705X_3 \quad (2)$$

The optimum conditions for maximum FAME yield were determined as: catalyst content = 1.5 wt.%, methanol/oil mole ratio = 9 and time = 40 min, according to these results.

Table 3. ANOVA results of statistical evaluation for the methyl ester yield; $R^2 = 0.95$, $R = 0.974$, $R^2(adj) = 0.926$

Sources of variations	Degree of freedom	Sum of squares	Mean square	*F*-value	Probability
Regression model	3	0.197495	0.065832	34.14	0.001
Error	5	0.009641	0.001928		
Corrected total	8	0.207136			

Four main fatty acids – palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and linolenic acid (C18:3) – were determined with noticeable quantities. As can be seen in Table 4, the highest FAME yields were belonged to oleic and linoleic acids. In order to see the effects of the reaction parameters on FAME yield of these fatty acids, oleic and linoleic acids were selected for the analysis. In Table 4, methyl ester yields of these fatty acids and design matrix obtained at different reaction conditions are presented.

Regression equation and ANOVA results were obtained as Eqs. (3) and (4) and Table 5 by using Minitab 14 software:

$$y_{(C18:1)} = 0.504 + 0.00395X_1 + 0.0144X_2 + +0.00165X_3,\ R^2 = 0.924 \quad (3)$$

$$y_{(C18:2)} = 0.176 + 0.00173X_1 + 0.00612X_2 + 0.00128X_3,\ R^2 = 0.913 \quad (4)$$

In the same way, it can be seen from the Eqs. (3) and (4) that the highest coefficient belonged to the catalyst/oil molar ratio among all the variables. It can also be indicated that C18:1 and C18:2 methyl ester yields were influenced positively from the reaction time and the methanol/oil ratio. The value of the determination coefficients were determined as $R^2 = 0.92$ and 0.91. These results showed that only about 8 and 9% of the total variation was not explained by the respective model. In conclusion, the optimum conditions for maximum FAME yield of C18:1 and C18:2 were found as catalyst content = 1.5 wt.%, methanol/oil mole ratio = 9 and time = 40 min, similar to previous results.

The effect of methanol/oil mole ratio

Transesterification reaction is an equilibrium reaction, lager amounts of alcohol can enhance the reaction to result in the equilibrium favorable to the FAME formation. Even though methyl ester production from any oil requires three moles of methanol for each mole of oil in theory, stoichiometrically, an excess amount of methanol is desirable for the completion of the reaction in practice [24]. In the ultrasound assisted process, alcohol reacts quite rapidly

Table 4. Methyl ester yields of fatty acids and design matrix obtained at different reaction conditions

Experiment No.	Methanol:oil mole ratio	Catalyst in oil share, wt.%	Time, min	X_1	X_2	X_3	C16=0	C18=1	C18=2	C18=3
1	3:1	0.5	20	-1	-1	-1	3.99	48.41	16.73	5.03
2			40	-1	-1	1	4.14	48.89	16.9	4.85
3		1.5	20	-1	1	-1	4.19	51.33	17.92	5.08
4			40	-1	1	1	4.31	51.79	18.08	5.22
5	9:1	0.5	20	1	-1	-1	4.22	49.92	17.21	5.02
6			40	1	-1	1	4.13	49.02	17.03	4.90
7		1.5	20	1	1	-1	4.14	51.68	17.95	5.05
8			40	1	1	1	4.27	52.96	18.82	5.32
9	6:1	1	30	0	0	0	4.16	49.96	17.41	4.97

Table 5. ANOVA results of statistical evaluation for the C18:1 and C18:2 methyl ester yield

Fatty acid	Sources of variations	Degree of freedom	Sum of squares	Mean square	*F*-value	Probability	R^2	*R*	R^2(adj)
C18:1	Regression model	3	0.00180548	0.00060183	20.22	0.003	0.92	0.959	0.878
	Error	5	0.00014884	0.00002977					
	Corrected total	8	0.00195432						
C18:2	Regression model	3	0.00033693	0.0001123	17.51	0.004	0.91	0.955	0.861
	Error	5	0.00003207	0.00000641					
	Corrected total	8	0.00036901						

due to an increased mass transfer in the presence of ultrasound. Ultrasound causes methanol to cavitate and disperse as nano-droplets into the oil, form a fine emulsion of methanol in oil, so the contact surface between reagents increases dramatically and consequently accelerates the reaction rate [15,25]. In order to investigate the effect of methanol/oil ratio on transesterification of microalgal oil in the presence of 0.5, 1 and 1.5 wt.% KOH catalyst in oil at different reaction times (20, 30 and 40 min), 3:1, 6:1 and 9:1 methanol/oil mole ratios were used, in this study. According to the results, it was found that, when methanol/oil ratio increased from 3:1 to 9:1, methyl ester yield increased. The highest methyl ester production was obtained with 9:1 methanol/oil mole ratio at the reaction time of 40 min and 1.5 wt.% KOH in oil. Similar results have been reported from various transesterification studies. Tamilarasan and Sahadevan have investigated ultrasonic assisted acid base transesterification of *Caulerpa peltata* oil. It was observed that an increase in the methanol to oil molar ratio concluded with an increase in methyl ester yield [21]. Tamilarasan *et al*. have studied ultrasound-enhanced rapid in situ transesterification of *Enteromorpha compressa*, marine macroalgae. Various quantity of methanol to algal biomass as 4:1, 4.5:1, 5:1, 5.5:1, 6:1 and 6.5:1) were applied to observe the effect of the methanol quantity on in situ transesterification process. It was reported that, 5.5:1 methanol to biomass ratio was suggested to be a suitable ratio for *in situ* transesterification process. For further increase of methanol ratio up to 6:1, no significant difference on the biodiesel yield was observed, and excess usage of methanol to biomass ratio above 6:1 decreased methyl ester yields [17]. Thanh *et al*. also carried out transesterification of canola oil by use of ultrasonic probe in the presence of KOH at the reaction time of 50 min. The highest methyl ester yield was obtained at methanol:oil mole ratio of 5:1 in their study [26]. Chuah *et al*. investigated empty fruit bunch doped with potassium hydroxide as a heterogeneous solid base catalyst for transesterification of rubber seed oil with methanol in ultrasonic batch system. It was found that up to 15:1 methanol:oil mole ratio, free fatty acid amount decreased with increasing amounts of the methanol:oil ratio. On the other hand, 15:1 methanol:oil ratio caused a reduction of methyl ester conversion and yield due to creating interference with the phase separation of glycerol which made an equilibrium of the reaction shift to reactants leading to difficulty of the separation process [27].

The effect of catalyst content

The effect of KOH in microalgal oil share was also studied, with various concentrations ranging from 0.5 to 1.5 wt.%. In comparison with sodium-based catalyst and methoxide catalyst, KOH was chosen due to its effectiveness on methyl ester conversion. The highest methyl ester yield was obtained with 1.5 w.t% KOH under the conditions of 9:1 methanol/oil mole ratio and reaction time of 40 min. As can be seen with the results, it was concluded that high yields of methyl ester were obtained with increasing catalyst/oil ratio when methanol/oil ratio was not varied. Stavarache *et al.* reported that, the yield of methyl ester increased with increasing catalyst quantity up to 1.0 wt.% NaOH. In the case of using 1.5 wt.% NaOH, methyl ester yield was very low due to the soap production in high quantity led to formation of gels in high quantity, and a high amount of esters remains trapped in the glycerin layer. However, when KOH was used, high yields were obtained even for 1.5% catalyst concentration. Potassium soap is softer, more soluble in water and does not make as much foam as sodium soap. The washing of esters when using KOH is easier and the yields of isolated product are higher. It was also reported that, in comparison between mechanical stirring and ultrasonic irradiation, ultrasonic irradiation showed higher efficiency than mechanical stirring [15].

Thanh *et al*. [24] studied the ultrasound assisted transesterification of triglycerides with methanol in the presence of KOH catalyst. It was reported that, the highest yield of FAME was achieved more than 98% after 20 and 25 min reaction time at the concentration of 1.5 and 1.0 wt.% KOH, respectively. Tamilarasan

and Sahadevan investigated the effect of catalyst concentration on methyl ester conversion with different catalyst concentrations of 0.25, 0.5, 0.75, 1, 1.25 and 1.5 wt.% NaOH. It was reported that, as the NaOH catalyst concentration increased, methyl ester conversion was found to be increased [21]. Chuah *et al.* also reported that, as catalyst, empty fruit bunch (EFB) embedded with KOH whose amount ranging from 0.5 to 2.5 wt.%, indicated the maximum FFA reduction was achieved when the catalyst concentration increased above 1.5 wt.% [27].

The effect of reaction time

The duration of the reaction has been established as one of the critical parameters for biodiesel production [17]. Reaction time is a crucial factor, which can not only determine the efficiency of biodiesel formation but also the quality of final products [19]. For this reason, choosing an optimum reaction time is required for both the completion of the reaction and produce high yields of methyl esters. Transesterification reactions were performed at 20, 30 and 40 min with different methanol:microalgal oil mole ratios and KOH catalyst/oil ratios to observe the effect of reaction time on methyl ester yield. An increase in reaction time resulted with higher methyl ester yields. It was observed that the highest methyl ester yield was obtained with the reaction time of 40 min under the conditions of 1.5% KOH and 9:1 methanol/oil mole ratio. The findings obtained from this experiment showed that the effect of reaction time on methyl ester yield were similar with the results of other studies in the literature. Kesgin et al. found that the highest formation of methyl ester was observed within 20 min at 4:1 and 5:1 mole ratios of alcohol:oil and indicated the ultrasound assisted transesterification reduces the processing time to 10-20 min when it was compared with the conventional transesterification [28]. In another study, transesterification of different vegetable oils with methanol, in the presence of potassium hydroxide as catalyst with low frequency ultrasound (40 kHz) was performed. In the experiments, effect of various reaction time (3-6-10-20-30-40-60 min) on conversion of vegetable oil to methyl ester was investigated and results showed that, increasing reaction time caused a decrease in the concentration of mono-, di- and triglycerides and increase in FAME concentration [25]. Avramovic *et al.* studied the ultrasound-assisted KOH catalyzed sunflower oil methanolysis kinetics, and results showed that FAME production increased fast up to 30 min reaction time, on the other hand, it was observed that after 30 min, FAME production increased slowly up to 60 min. As expected, the initial rate of the reaction was slow due to mass transfer limitation. As the transesterification proceeded, the drops of methanol were disintegrated by the effect of the ultrasound waves and they were stabilized by monoglycerides, diglycerides and soaps. This was resulted in an increase of the interfacial area available for mass transfer [29]. Suganya *et al.* have studied ultrasound-enhanced rapid *in situ* transesterification of *E. compressa* and investigate the effect of time on the process, the reaction time ranging from 10 to 120 min which was maintained with the optimized parameters of 10% H_2SO_4 concentration, 30 vol.% of tetrahydrofuran and 5.5:1 methanol to biomass ratio. Under these conditions, they have achieved the highest yield of 98.89% in 90 min of reaction time [17]. In the study of Hindryawati and Maniam, transesterification experiments were carried out with different reaction times varying 0-80 min. It was found that the highest methyl ester yield was obtained at the reaction time of 30 min under the conditions of 3 wt.% catalyst and methanol to oil molar ratio of 9:1 at 55 °C. It was reported that any further time increase caused no remarkable variations in the content due to equilibrium [30].

Fatty acid profile in FAME samples

According to the GC results, it was found that there were four main fatty acids as palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and linolenic acid (C18:3). In all samples, oleic acid was determined as the highest amount of fatty acid methyl ester found in biodiesel. It constituted more than 50% of the FAME. Palmitic, linoleic and linolenic acid content were approximately determined as 4, 17 and 5%, respectively. Trace amounts of other fatty acids such as C16:1, C18:0, C20:0 and C20:1 were also determined, however they could not be calculated with these results. Fatty acid profile of biodiesel is a very important measure for determining the quality of the product that will be used as fuel. Therefore, achieving high quality biodiesel depends on suitable fatty acid composition [31]. In order to decrease oxidative stability and cold flow problems, it is required to have low levels of polyunsaturated and low levels of saturated FAs. Studies showed that, monounsaturated fatty acids of palmitoleic acid (16:1) and oleic acid (18:1) can provide an appropriate balance between oxidative stability and cold flow. Besides that, the five most common C16–C18 fatty acids, as palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids are necessary for a good quality biodiesel product. Also, it has been indicated that C16:1 and C18:1 fatty acids (palmitoleic acid and

oleic acid, respectively) are the most favorable fatty acids for biodiesel production [32]. According to this, it can be said that the obtained biodiesel possessed suitable fatty acids convenient for biodiesel production. In literature, it was indicated that high linolenic acid content has negative effects on oxidative stability and cold flow properties of biodiesel. In this study, linolenic acid content was found to be only around 5% of total FAME.

Properties of methyl esters

Important properties that affect biodiesel usage as a fuel should be determined in order to evaluate its potential as a substitute for diesel fuel. Determination of these properties was carried out with characterizing the obtained product with its acid value, iodine value, density, methyl ester content, water content and viscosity. Experimental procedures were applied in according to biodiesel standards. Methyl ester which was obtained under the conditions of methanol-oil mole ratio: 9:1; 1.5% KOH; and t = 40 min used to perform characterization experiments. Ester, monoglyceride, diglyceride and triglyceride contents of the obtained product were determined as 99.56, 0.119, 0.021 and 0.031%, respectively. Characterization of methyl ester was carried out according to experimental procedures of EN 14104, EN 14111, EN ISO 3679, EN ISO 12937 and EN ISO 3104. Acid value, iodine value and density of algal biodiesel were found as 0.36 mg KOH/g, 86.40 g I_2/100 g and 0.868 g/cm^3, respectively. Water content and viscosity of biodiesel were measured as 0.014% and 4.7 mm^2/s, respectively. According to the commercial biodiesel standards, values of the properties of algal biodiesel were in the range of acceptance criteria as compared with commercial biodiesel standards. As a result, this product can be evaluated as a fuel.

CONCLUSIONS

In this study, *C. prototheco*ides oil was trancesterified by using ultrasound assisted method, and transesterification parameters on methyl ester production were investigated. Experimental and statistical results showed that the catalyst/oil ratio is the most effective parameter on methyl ester yield. Similarly, statistical results showed that effect of catalyst/oil ratio on methyl ester yield of oleic and linoleic acid is the highest in comparison with methanol/oil ratio and reaction time. Oleic acid methyl ester was found as more than 50% of the FAME, which was the highest amount of FAME in algal biodiesel in all samples. Although ultrasound assisted trancesterrification is a very promising way for biodiesel production, there are only a few studies on biodiesel production from microalgal oils by using this method. Besides that, ultrasound assisted transesterification does not presently seem economically viable for large scale production of algal biodiesel. Therefore, there is still need for innovations for both high productivity and green productions.

ACKNOWLEDGMENTS

The authors acknowledge financial support from the Yıldız Technical University Scientific Research project (2013-07-04-KAP08) provided for this work.

REFERENCES

[1] W.N.M.W. Ghazali, R. Mamat, H.H. Masjuki, G. Najafi, Renewable Sustainable Energy Rev. **51** (2015) 585-602

[2] BP, BP Statistical Review of World Energy June 2015 64th ed., https://www.bp.com/content/dam/bp/pdf/energy--economics/statistical-review-2015/bp-statistical-review--of-world-energy-2015-full-report.pdf (accessed 30 July 2016)

[3] International Energy Agency, Oil, https://www.iea.org/ /aboutus/faqs/oil/ (accessed 30 July 2016)

[4] M. Koberg, M. Cohen, A. Ben-Amotz, A. Gedanken, Bioresour. Technol. **102**(5) (2011) 4265-4269.

[5] D.C. Kligerman, E.J. Bouwer, Renewable Sustainable Energy Rev. **52** (2015) 1834-1846

[6] A. Singh, P.S. Nigam, J.D. Murphy, Bioresour. Technol. **102**(1) (2011) 26-34

[7] R. Halim, M.K. Danquah, P.A. Webley, Biotechnol. Adv. **30**(3) (2012) 709-732

[8] K. Soratana, W.F. Harper Jr., A.E. Landis, Energy Policy **46** (2012) 498-510

[9] S. Goto, M. Oguma, N. Chollacoop, in EAS-ERIA Biodiesel Fuel Trade Handbook, S. Goto, M. Oguma, N. Chollacoop, Eds., ERIA, Jakarta, 2010, pp. 27-62

[10] X. Feng, T.H. Walker, W.C. Bridges, C. Thornton, K. Gopalakrishnan, Bioresour. Technol. **166** (2014)17-23

[11] D. Özçimen, M.Ö. Gülyurt, B. İnan, in Biodiesel - Feedstocks, Production and Applications, Z. Fang, Ed., InTech, Rijeka, 2012, pp. 25-57

[12] A.P. Vyas, J.L. Verma, N. Subrahmanyam, Fuel **89**(1) (2010) 1-9

[13] D. Özçimen S. Yücel, in Biofuel's Engineering Process Technology, M.A. Bernardes, Ed., InTech, Rijeka 2011, pp. 353-384

[14] S. Gryglewicz, Bioresour. Technol. **70**(3) (1999) 249-253

[15] C. Stavarache, M. Vinatoru, R. Nishimura, Y. Maeda, Ultrason. Sonochem. **12**(5) (2005) 367-372

[16] E.A. Ehimen, Z. Sun, G.C. Carrington, Procedia Environ. Sci. **15** (2012) 47-55

[17] T. Suganya, R. Kasirajan, S. Renganathan, Bioresour. Technol. **156** (2014) 283-290

[18] A. Guldhe, B. Singh, I. Rawat, F. Bux, Chem. Eng. Res. Des. **92**(8) (2014) 1503-1511

[19] G. Ma, W. Hu, H. Pei, L. Jiang, M. Song, R. Mu, Energy Convers. Manage. **90** (2015) 41-46

[20] E. Martinez-Guerra, V. G. Gude, A. Mondala, W. Holmes, R. Hernandez, Appl. Energy, **129** (2014) 354-363

[21] S. Tamilarasan, R. Sahadevan, Fuel **128** (2014) 347-355

[22] M.Ö. Gülyurt, D. Özçimen, B. İnan, Int. J. Mol. Sci. **17**(4) (2016) 579

[23] D. Özçimen, J. Biobased Mater. Bioenergy **7**(4) (2013) 449-456

[24] L.T. Thanh, K. Okitsu, Y. Maeda, H. Bandow, Ultrason. Sonochem. **21**(2) (2014) 467-471

[25] C. Stavarache, M. Vinatoru, Y. Maeda, Ultrason. Sonochem. **14**(3) (2007) 380-386

[26] L.T. Thanh, K. Okitsu, Y. Sadanaga, N. Takenaka, Y. Maeda, H. Bandow, Bioresour. Technol. **101**(14) (2010) 5394-5401

[27] L.F. Chuah, M.M. Amin, S. Yusup, A. Bokhari, J.J. Klemeš, M.S. Alnarabiji, J. Cleaner Prod. (2016) (in-press)

[28] C. Kesgin, S. Yücel, D. Özçimen, P. Terzioğlu, A. Attar, Int. J. Green Energy, (2014) in press.

[29] [J. M. Avramović, O. S. Stamenković, Z. B. Todorović, M. L. Lazić, V. B. Veljković, Chem. Ind. Chem. Eng. Q. **18**(1) (2012) 115-127

[30] N. Hindryawati, G.P. Maniam, Ultrason. Sonochem. **22** (2015) 454-462

[31] A.F. Talebi, S.K. Mohtashami, M. Tabatabaei, M. Tohidfar, A. Bagheri, M. Zeinalabedini, H. H. Mirzaei, M. Mirzajanzadeh, S. M. Shafaroudi, S. Bakhtiari, Algal Res. **2**(3) (2013) 258-267

[32] M. Song, H. Pei, W. Hu, G. Ma, Bioresour. Technol. **141** (2013) 245-251.

DIDEM ÖZÇIMEN
M. ÖMER GÜLYURT
BENAN İNAN

Department of Bioengineering, Faculty of Chemical and Metallurgical Engineering, Yıldız Technical University, Davutpasa Campus, Istanbul, Turkey

NAUČNI RAD

OPTIMIZACIJA PROIZVODNJE BIODIZELA IZ ULJA *Chlorella protothecoides* ULTRAZVUKOM POTPOMOGNUTOM TRANSESTERIFIKACIJOM

Sve je veći interes za biodizelom kao alternativnim gorivom za dizel motore zbog visokih cena nafte i ekoloških problema uzrokovanih na masivvelikom emisijom gasova sa efektom staklene bašte. Danas je mikroalgalna biomasa postala obećavajuća sirovina za dobijanje biodizela. Međutim, tradicionalna proizvodnja biodizela iz mikroalgi troši mnogo energije i rastvarača. Neophodno je koristiti alternativnu metodu koja može smanjiti potrošnju energije i alkohola i uštedeti vreme. U ovom radu, istraživan je uticaj odnosa metanol:ulje i vremena trajanja reakcije na prinos metil estara masnih kiselina (MEMK) u proizvodnji biodizela iz ulja Chlorella protothecoides pomoću ultrazvukom-potpomognute transesterifikacije metanola i kalijum-hidroksida kao katalizatora. Najveća prinos metil-estra dobijen je pod uslovima molskog odnosa metanol:ulje od 9:1, odnosa katalizator:ulje od 1,5% kalijum-hidroksida i reakcionog vremena od 40 min. Takođe, utvrđeno je da je molski odnos katalizator:ulje statistički najvažniji faktor. Rezultati pokazuju da ultrazvukom-potpomognuta transesterifikacija može biti efikasna i isplativ alternativni način za proizvodnju biodizela.

Ključne reči: biodizel dobijen iz algi, Chlorella protothecoides, metil-estar, transesterifikacija, ultrazvuk.