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Production of high purity biodiesel through direct saponification of wet biomass of *Chlorella protothecoides* in a low cost microwave reactor: Kinetic and thermodynamic studies

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Abstract−We studied production of biodiesel from microalga *Chlorella protothecoides* (SAG 211-10 C) through direct saponification of its wet biomass (70% moisture) in a microwave reactor using ethanolic potassium hydroxide. The resulting soap was precipitated by "common ion effect" using saturated solution of potassium chloride and subjected to simultaneous acidulation and esterification to form biodiesel. The optimum parameters for saponification were: Temperaure-60 °C, Ethanol to dry biomass ratio (ml/g)-80 : 1, concentration of KOH-0.5%, microwave power-450 W; and for esterification they were Temperature-60 °C, wt% of sulfuric acid-2.5%, molar ratio of methanol to fatty acids-70 : 1, microwave power-450 W. The kinetics and thermodynamics of saponification and esterification were investigated. Both reactions were found to follow pseudo-first-order kinetics. Activation energies were determined as 14.177 kJ/mol and 17.234 kJ/mol for saponification and esterification, respectively. The final biodiesel yield and purity were 98.74% and 94.83%, respectively.

Keywords: Biodiesel, *Chlorella protothecoides*, Common Ion Effect, Microwave Reactor, Direct Saponification

INTRODUCTION

The conventional approach to biodiesel production from microalgae involves different processes, including microalgae cultivation, harvesting of biomass and drying, oil extraction, and biodiesel conversion [1]. Among these, biomass drying, oil extraction and biodiesel conversion processes are critical for the industrial-scale production of microalgal biodiesel. Especially, drying microalgae after harvesting requires high energy and accounts for 20-30% of the total cost of biodiesel production [2]. One of the alternatives to overcome these limitations is the 'in situ' transesterification method, in which the lipids of wet algae are simultaneously extracted and converted to Fatty acid Methyl esters (FAME). Since the *in situ* approach integrates extraction and conversion in one step, it eliminates the need for drying algae biomass and the isolation and refining of lipid before converting it to biodiesel. This could lead to a reduction in the cost of biodiesel [3]. Moreover, besides serving as a reactant in the *in situ* process, methanol used in this process weakens the cellular and lipid body membranes to facilitate the FAME conversion [4]. Several studies have reported *in situ* transesterification of wet algal biomass [5-8]. However, this technique suffers from a high requirement of methanol and sulfuric acid, which must be avoided due to requirement of a large reactor and its corrosion by sulfuric acid. Also, since, formation of FAME is a reversible reaction, the presence of water can hydrolyze biodiesel back to methanol and free fatty acids [9]. A solution to this problem is direct saponification of wet biomass, which is faster and cheaper than lipid extraction [10]. Recently, some authors have reported a two-step process: direct saponification of wet biomass of microalgae, followed by extraction and esterification of fatty acids [5,11-13]. However, high purity biodiesel (96.5%) biodiesel) was obtained by Pena et al. [12] alone. The presence of unsaponificable matter in microalgae decreases the purity of biodiesel. Hence, a simple and effective separation process is required to separate them. This leads to greater purity of biodiesel.

In this regard, we describe here direct saponification of wet algae biomass of *Chlorella protothecoides* using ethanolic KOH in microwave reactor to produce salts of fatty acids (soaps). The rationale behind selecting microwave irradiation was that more lipids are extracted in solvent phase in microwave extraction than conventional heating due to volumetric heating, which ultimately increases the yield of biodiesel. Effects of operating conditions, which influence the yield of saponification, such as temperature, microwave power, reaction time, ethanol to dry biomass ratio (ml/g) and concentration of KOH in ethanol, were evaluated.

The second novelty of the present work lies in the methodology adopted for the separation of soaps from unsaponificable matter. Conventionally, in soap production, after saponification, brine is added to precipitate soap in the form of 'soap curds' which rise to the top of the soap kettle. The remaining solution containing salt, glycerin and excess alkali, impurities and coloring matter is called as "spent lye" and is drawn from the bottom of the kettle [14]. This process facilitates separation of soaps from rest of the solution in one step. In the case of direct saponification of wet algal biomass, the reaction mass will be a complex suspension comprised of soaps, unconverted lipids, unsaponificable matter (protein, carbohydrates, chlorophylls, sterols, waxes etc.) and ruptured algae cells. To isolate soaps from this solution, adequate volume of saturated

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KCl solution can be added which precipitates soaps due to 'common-ion effect'. The precipitate can be further acidulated to form fatty acids, which further can be converted to FAME. This strategy was adopted in the present work to achieve higher purity of biodiesel.

EXPERIMENTAL SECTION

1. Algae Strain Collection and Culture Condition

The original strain of *C. protothecoides* (SAG 211-10 C) was obtained from Sammulung von Algenkulturen (SAG), Germany, and maintained on agar slants containing BG11 medium consisting of (g/L): $NaNO_3$-1.5, $K_2HPO_4 \cdot 3H_2O$-0.04, $KH_2PO_4 \cdot 3H_2O$-0.2, EDTA-0.0005, Ferric ammonium citrate-0.005, citric acid-0.005, Na_2CO_3-0.02 and 1 mL of trace metal solution. The trace metal solution contains (g/L): H_3BO_3-2.85, $MnCl_2 \cdot 4H_2O$-1.8, $ZnSO_4 \cdot 7H_2O$-0.02, $CuSO_4 \cdot 5H_2O$-0.08, $CoCl_2 \cdot 6H_2O$-0.08 and $Na_2MoO_4 \cdot 2H_2O$-0.05. The pH of the medium was adjusted to 6.8. Stock cultures were inoculated into 100 mL medium in 250 mL Erlenmeyer flasks. The flasks were then incubated at 24 °C in a rotary shaker and agitated at 120 rpm. After seven days, the algal biomass was harvested by centrifugation (REMI c-24 bl) at 10,000 rpm for 10 min to obtain a wet paste containing 70% moisture.

2. Construction of Microwave Reactor

A domestic kitchen oven (Samsung, Maximum power 800 W) was modified to a microwave reactor. The pictorial view and schematic diagrams of microwave reactor used in the present are shown in Figs. 1, 2 and 3, respectively. The details of specifications of its components are in Table 1. On the top portion of the oven, a circular cut of diameter 25 mm was made and fitted with a steel stud

Fig. 1. Experimental setup of microwave reactor.

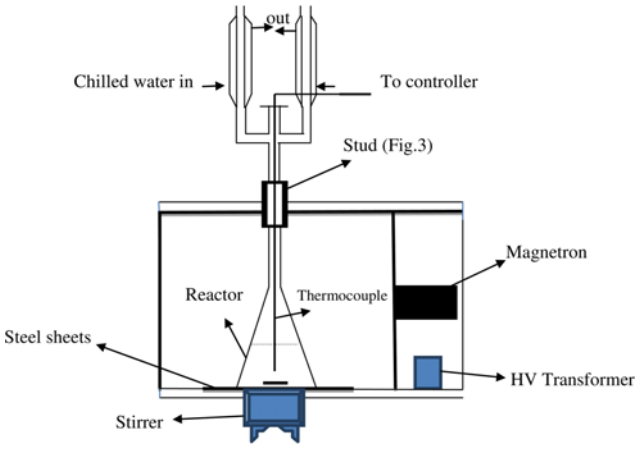

Fig. 2. Construction details of microwave reactor.

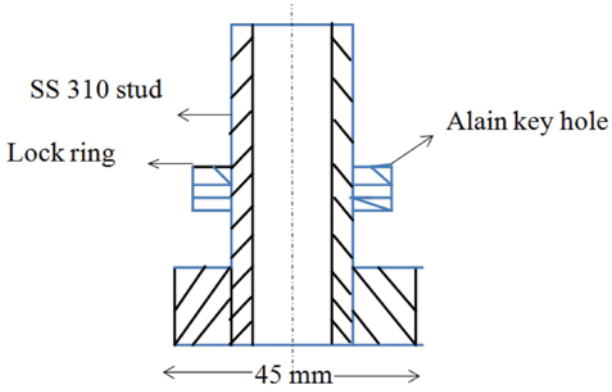

Fig. 3. Details of stud.

Table 1. Details of specification of components in microwave reactor

S. no.	Code	Components	Make	Specifications
1	S1	Control switch	SALZER	1 POLE , 230 VAC
2	V	Voltmeter	UNIVERSAL	1 PHASE
3	PR	ON/OFF PID Controller	OMRON	E5CWL
4	TC	Thermocouple	OMEGA	J-TYPE
5	S2	Heater controller switch	TECHNIQUE	SINGLE POLE
6	F	Fuse - 2 AMP	L-MAX	TB KUDF
7	RL	Relay	LOCAL	DOUBLE POLE
8	L1,2	Indicator lamps	L-MAX	Local
9	F	Cooling fan	LOCAL.	1 PHASE
10	MW	Microwave oven	SAMSUNG	800 W
11	ST	Magnetic stirrer	REMI	230 VAC

and support ring. The support ring was tightened with an Alain key provided on the side. This is for arresting the leakage of microwave to outside environment. A 500 ml borosilicate glass beaker with a flat bottom was used as reactor. Through the stud fitting, the neck of a tri end assembly was inserted. A thermocouple (Pt-100) was used to measure the liquid temperature. The thermocouple was connected to the temperature controller (PR). The precision of temperature control was ±0.1 °C. The magnetic stirrer action was independent of other circuits. The magnetic stirrer was placed below the microwave oven after cutting the bottom plate of the cavity to the size of the top plate of stirrer. On the top of the bottom plate, a steel sheet was provided to close the opening. All the sharp edges were camouflaged with aluminium tape to avoid any interaction of microwave field with magnetic field.

3. Circuit Diagram

The circuit diagram is shown in the Fig. 4. Switch S1 is provided to control the power to the control panel. This is directly connected to the main supply. When S1 is ON, the power control panel will energize and the control light IL and temperature controller will be ON. Another power supply from the microwave is fed to the control panel for power control to the microwave. This power can be ON from the microwave MW ON switch. After switching on the microwave power, the controller is in run mode and S2 switch is on. Then, the contact relay (RL) gets activated and the power flows to the HV transformer. In turn, the magnetron is activated. Indicator lamp IL2 will be on and the voltmeter will show the power. Once the reactor temperature is above the set temperature, the temperature controller (PR) will cut off the power to the microwave through the relay.

4. Direct Saponification of Wet Algae Biomass

In all trials, 10 g of wet algae paste with 70% water was transferred to microwave reactor. Desired quantity of known concentration of ethanolic KOH was added. The reaction temperature and time were set at predetermined values. Stirring speed in all trials was maintained at 700 rpm. Since the vapor generation rate was high due to microwave heating, chilled water was passed through condensers for efficient condensation. Following saponification, the reaction mixture was cooled to room temperature and biomass residue was separated by centrifuging at 10,000 rpm. It was washed twice with 20 ml ethanol to recover adhering solution which was pooled with supernatant. The final volume of supernatant was measured and a calculated amount of saturated KCl was added and the solution was stirred at 400 rpm in magnetic stirrer for 5 min. The solution was then allowed for phase separation. A white soap precipitate floating at the top was formed, which was separated by filtration using Whatman filter 1 and rinsed twice with 25 ml hexane to extract the unsaponificable matter adhering to soap curds. Finally, the soap curd was dried in an oven for 2 h to remove traces of hexane.

5. Acidulation of Soaps and Esterification of Fatty Acids

0.3 g of soap was dissolved in known quantity methanol and sulfuric acid. The solution was transferred to a microwave reactor

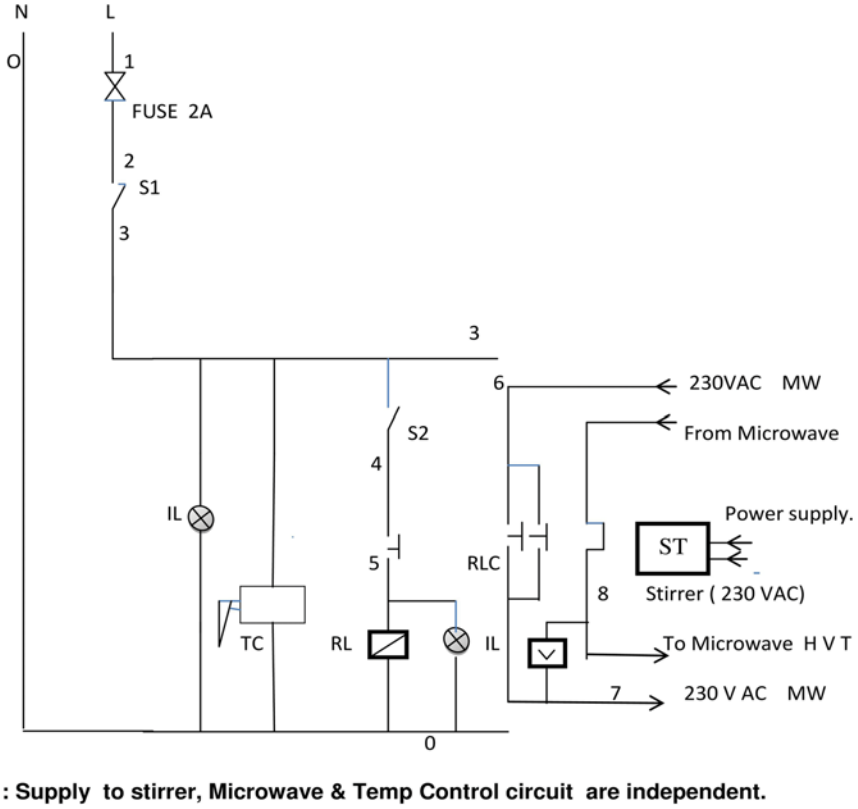

Fig. 4. Circuit diagram of microwave reactor.

S1. Control switch	S2. MW power ON/OFF	RL. Relay	TC. Thermocouple	F. Fuse 2 A
V. Analogue voltmeter	PR. Temp. controller	IL. Indicator lamp	ST. Stirrer	RLC. Relay contact

and esterification was carried out for preset time. Following the reaction, 100 ml hexane was added and the mixture was shaken for 45 min at 700 rpm to extract biodiesel, and then allowed to settle for 1 h. The top hexane phase containing biodiesel was separated and evaporated in a rtary vacuum evaporator (Superfit™, Rotavap, Model: PBV-7D) to remove solvent and recover biodiesel. The GC analysis of FAMEs was carried out. Influence of parameters such as temperature, reaction time, concentration of sulfuric acid and level of microwave power on yield of biodiesel was analyzed.

6. Analysis

6-1. Total Lipid and Esterificable Fatty Acids Content in Algal Biomass

Total lipids were estimated following the protocol of Bligh and Dyer [15]. To a 15 ml vial containing 300 mg algal biomass, 2 ml methanol, and 4 ml chloroform were added. The mixture was agitated in a cyclomixer (Model: CM 101, REMI) for 2 min. 1 ml of chloroform was again added and the mixture was shaken vigorously for 1 min. 1.8 ml of distilled water was added and the mixture was mixed in a vortex again for 2 min. The two layers were separated by centrifugation for 15 min at 10,000 rpm. The lower layer was filtered through Whatman No. 1 filter paper into a previously weighed clean vial (W_1). The process was repeated three times. All the chloroform phases were collected together and evaporated in a rotary vacuum evaporator (Superfit™, Rotavap, Model: PBV-7D). The final weight of vial with lipids was noted (W_2). The lipids obtained were calculated by subtracting W_1 from W_2, and was expressed as % dcw.

For the determination of total esterificable fatty acid (EFA) content, the lipids were redissolved in 10 ml methanol and 2% concentrated sulfuric acid as catalyst and heated in thermostat at 90 °C for 2 h. After the reaction, the mixture was cooled to room temperature. 5 ml deionized water and 8 ml hexane were added to form two phases. The upper phase containing FAMEs was transferred to 10 ml centrifuge vials and analyzed by gas chromatography.

6-2. FAME Content in Biodiesel

Agilent 7890B equipped with HP-5 column (30.0 m×0.32 mm×0.25 μm) was used with FID detector. The injector and detector temperature was set at 250 °C and N_2 was used as carrier gas at a flow rate at 2 ml/min. The oven temperature was programmed to start at 60 °C and increased to 175 °C at a rate of 25 °C min^{-1} and then to 240 °C at a rate of 4 °C min^{-1} and held constant for 20 min^{-1}. 1 mg of internal standard solution of methyl heptadecanoate was added. The analysis was performed by injecting 1 ml of sample solution into the gas chromatograph. FAME was quantified by comparing the peak areas between the samples with those of the standard compounds. The percentages of each peak/FAME were calculated, and based on these values, the FAME conversion was calculated.

6-3. Acid Value

5 ml of sample was transferred to 250 ml conical flask. 50 ml of freshly neutralized hot ethyl alcohol and 1 ml of phenolphthalein TS were added to it. The mixture was boiled for 5-10 min and titrated against KOH as hot as possible until the pink color persisted for at least 30 s. Acid value of sample was calculated as,

$$\text{Acid value (mg KOH/g lipid)} = (56.1 \times V_{KOH} \times N_{KOH}) / (\text{weight of lipid in sample in g}) \quad (1)$$

where, V_{KOH} is volume of KOH run down (ml) and N_{KOH} is normality of KOH (0.1 N)

7. Yield of Saponification, Esterification Reactions and Purity of Biodiesel

$$\text{Yield of saponification reaction} = \frac{\text{g of soaps formed}}{\text{g of EFA in algal biomass}} \times 100 \quad (2)$$

$$\text{Yield of FAMEs} = \frac{\text{g of FAMEs in biodiesel}}{\text{g of EFA in algal biomass}} \times 100 \quad (3)$$

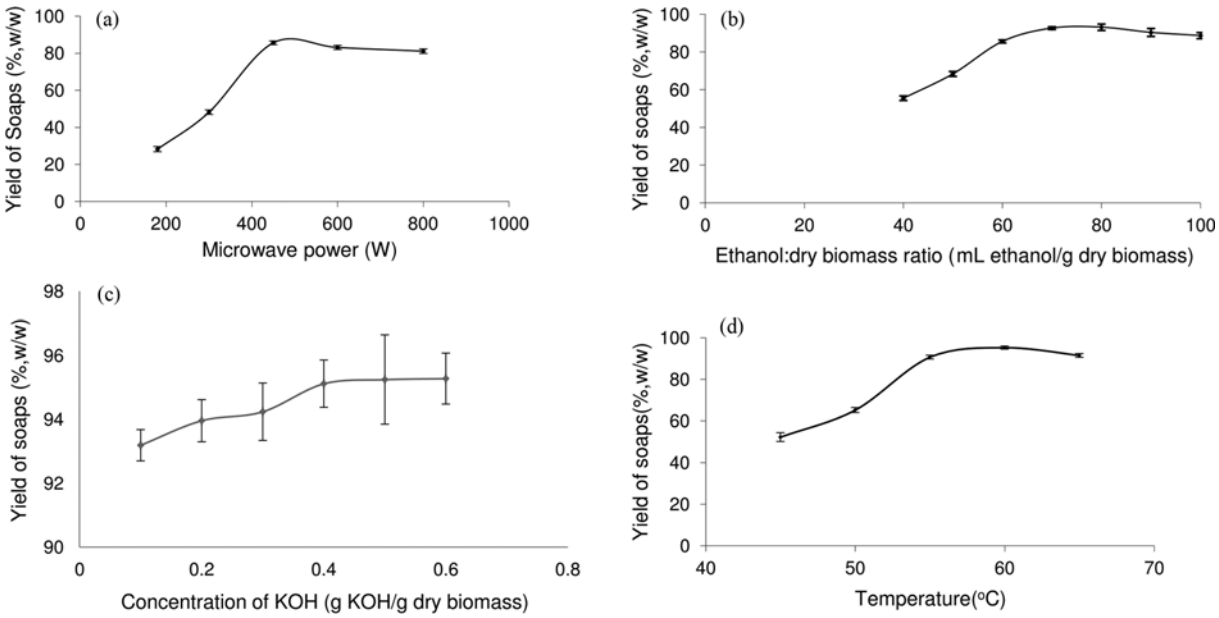

Fig. 5. Effect of parameters on yield of saponification ((a) Effect of microwave power, (b) Effect of ethanol:dry biomass ratio, (c) Effect of temperature, (d) Effect of concentration of KOH).

$$\text{Purity of biodiesel} = \frac{\text{g of FAMEs in biodiesel}}{\text{g of biodiesel}} \times 100 \quad (4)$$

RESULTS AND DISCUSSION

1. Factors Influencing Saponification

1-1. Effect of Microwave Power

Experiments were conducted at five microwave power levels (180 W, 300 W, 450 W, 600 W and 800 W). As shown in Fig. 5(a), the yield of soaps was meager at 180 W (28.25%), which may be due to lower microwave efficiency at lower power level, while optimum power level was 450 W (85.65%, w/w). Theoretically, with increase in applied microwave power, yield of soaps should increase. Two factors may be responsible for this. First, microwave heating extracts more lipids than conventional heating due to increased intracellular heating rates that result in pressurized effects, which ruptures cell membrane, releasing the lipids. In the present work, wet algae of 70% water content was used along with ethanol. The presence of two polar components may be responsible for intracellular heating rates [16]. The second reason for high yield of soaps at high microwave power can be explained by considering the Arrhenius equation, $K=Ae^{-\Delta G/RT}$. The free energy of saponification (ΔG) decreases at higher microwave power [17], resulting in enhanced yield of soaps. Thus, increasing microwave power should actually increase quantity of lipids extracted, which in turn increases the yield of soaps. However, with increase in microwave power, solvent evaporation losses are high, which results in reduction of available solvent for extraction. Thus, it can be concluded that there are two opposing driving forces which determine the yield of soaps. In the present work, an effective cooling operation was adopted using chilled water at 5 °C. Still, at 600 W and 800 W, we observed a reduction in the soap yield (83.14% and 81.16%, respectively), which can be attributable to higher solvent losses at high power of microwave.

1-2. Effect of Ethanol to Dry Biomass Ratio

Fig. 5(b) shows the effect of increasing ethanol/wet biomass ratio on conversion of saponification reaction. Ethanol, a cheap and safe solvent, has some affinity to the lipid complex, especially under high temperature [17,18]. Fajardo et al. [18] used an ethanol-based method for extracting lipids from drying microalga *Phaeodactylum tricornutum* efficiently. Gonzalez et al. [11] conducted direct saponification of wet microalga *P. tricornutum* UTEX 640 using 2.09 ml ethanol (96%) mixed with 0.4 g KOH/g of wet biomass. Under these conditions, the fatty acid yield was 87%. However, in these works a microwave effect was absent and hence, the mechanism of lipid extraction was basically by diffusion of ethanol through cell membrane. On the other hand, under microwave effect, intracellular lipids are directly released into ethanol from ruptured cells. For the membrane-associated lipids which are strongly linked to proteins, ethanol disrupts lipid-protein interactions and forms a hydrogen bond with them [19]. Then the lipids are dissolved in ethanol and react with KOH. In the present work, the yield of soaps increased from 55.55% to 93.19% when the ratio of ethanol to dry biomass was increased from 40 : 1 to 80 : 1 ml ethanol/g dry biomass (Fig. 5(b)). Surprisingly, further increase in ratio to 90 : 1 led to decrease in yield. Too large quantity of ethanol absorbs microwave irradiation preferentially and decreases the amount of radiation available to algal cells. Hence, both from economy and efficiency point of view, an adequate ratio of ethanol to dry biomass was established as 80 : 1.

1-3. Effect of KOH Concentration in Ethanol

Basically, potassium hydroxide acts on cell wall of microalgae and dissolves the phospholipids. Further, it reacts with lipids to produce soaps. Thus, increasing KOH concentration in ethanol should naturally increase yield of soaps. However, in the present work, the effect of microwave irradiation was sufficient to break down the cell walls and ethanol could easily dissolve them. Thus, the role of KOH was primarily reflected in saponification. The saponification value of *C. protothecoids* lipids in the present work was 261.25±1.2 mg KOH/g lipids. Thus, stoichiometrically, a concentration of 0.03 g KOH/g dry biomass was sufficient (% esterifiable/saponifiable lipids in microalgae was 12.82 wt%). However, excess KOH was used in the present work to ensure complete saponification. But, using a higher dosage of KOH increases the pH of soaps formed and hence increases the quantity of sulfuric acid required to acidulate it. Thus, to arrive at an optimum KOH concentration, experiments were conducted with varying concentrations of KOH in ethanol. As illustrated in Fig. 5(c), 0.5 g KOH/g dry biomass was found to be adequate. Maximum yield of 95.25% was noticed at this level of concentration of catalyst.

1-4. Effect of Temperature

To study the effect of temperature on yield of soaps, saponification experiments were conducted in the range of 45 °C-65 °C and the results are depicted in Fig. 5(d). At 45 °C, relatively lower yield (52.23%) was observed. At low reaction temperature, thermal effect was not sufficient to break the rigid cell-wall of dry algal biomass for better percolation of the solvent, and this might have led to lower lipid yield. Also, molecular diffusion was slow and not sufficient activated molecules were available to promote the reaction. The yield of soaps increased with increasing temperature and a highest yield of 95.25% was observed at 60 °C. This can be attributed to two factors. First, the solubility of lipids in ethanol increases with increasing temperature. The second possible reason is that the temperature enhances the kinetics of the lipid conversion through saponification. With rise in temperature, localized "hot spots" are formed which provide enough energy for the molecules to react. Wahidin et al. [20] reported that with the same treatment time, the lipid yield increased by 30% when the temperature increased from 58.1±3.42 °C to 83.8±2.7 °C. In the present work, at 65 °C a decrease in yield of soaps was noticed (91.14%), which may be due to a decrease in available quantity of ethanol because of vaporization (Nearly 38.5% of ethanol losses were observed at 65 °C).

2. Simultaneous Acidulation and Esterification

2-1. Effect of Temperature

The reaction mixture for esterification consists of methanol, sulfuric acid and fatty acids. Among these, methanol, which contains an -OH group, is a strong microwave absorption material. The dipole rotates at a high speed under the changing electrical field, which leads to molecular friction and local superheating, which accelerates the reaction to complete faster [21]. Lokman et al. [22] reported that no significant effect of temperature was found on esterification of palm fatty acid distillate, and 55 °C was found to

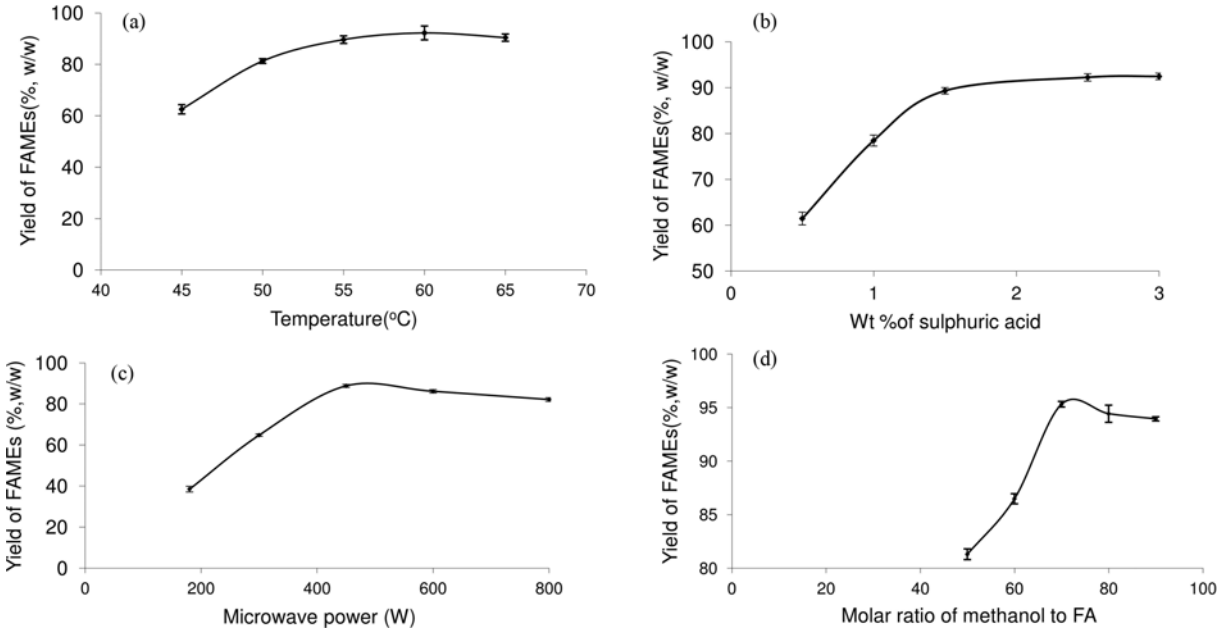

Fig. 6. Effect of parameters on yield of esterification ((a) Effect of temperature, (b) Effect of concnetration of sulfuric acid, (c) Effect of microwave power, (d) Effect of molar ratio of methanol on FA).

be optimum temperature for safer operation. In the present work, to evaluate the effect of temperature on FAME yield, experiments were conducted in the temperature range of 45 °C-65 °C. The results are depicted in Fig. 6(a). It can be observed FAME yield sharply improved from 62.54% at 45 °C to maximum value of 90.41% at 60 °C. However, at 65 °C, a decline in yield was observed to 92.25%, which may be due to evaporation of methanol at its boiling point, thus decreasing the amount available for reaction (Nearly 44.16% of methanol losses were observed).

2-2. Effect of Sulfuric Acid Concentration in Methanol

Sulfuric acid performs two actions: i) Decreases the pH of soaps to acidic conditions and thus transforms soaps to fatty acids, and ii) catalyzes the esterification of fatty acids. In the present work, various concentrations of sulfuric acid in methanol were tested and results are depicted in Fig. 6(b). At 0.5%, relatively lower yield of FAME was observed. This may because of insufficient concentration of sulfuric acid. The yield of FAME increased with increasing concentration of sulfuric acid and reached 92.25% at 2.5%. At 3%, the yield marginally improved to 92.46%. For a practical operation, high concentration of sulfuric should be avoided as it is corrosive. Therefore, a concentration of 2.5% was assumed to be sufficient.

2-3. Effect of Microwave Power

Microwave power affects the rates of reaction primarily be reducing its activation energy due to dipolar polarization effect. This is achieved due to molecular level interaction of the microwaves in the reaction mixture, resulting in dipolar rotation and ionic conduction. To arrive at appropriate power level, five trails were conducted at varying power levels. A low yield (38.47%) observed at 180 W was attributable to extremely poor heating rates and longer time required to attain the reaction temperature (Fig. 6(c)). With 100% power level, reactants attained set temperature quickly. But, more power losses were observed. Similar to saponification reaction, 450 W was found to be an adequate level of microwave power. Increasing the power beyond this level had a negative effect on yield of FAME.

2-4. Effect of Methanol to Fatty Acids Ratio

As the production of biodiesel is a reversible reaction, excess methanol is essential to accelerate the reaction to forward direction and support the synthesis of biodiesel. Concentration of methanol plays a vital role in esterification of fatty acids. A high methanol-to-fatty acids ratio improves FAME yield due to increased contact area between increases the contact area between them. Further, since esterification is a reversible reaction, a high methanol concentration shits the reaction to product side. Further, methanol is polar solvent and good absorber of microwave irradiation (Loss factor=0.59 at 2.45 GHz), which absorbs most of the microwave effect in its entire spectrum to produce localized superheating in the reactants and assists the reaction to complete faster [23]. Hence, effect of increasing ratio of methanol to fatty acids was positive on yield of biodiesel as shown in Fig. 6(d). However, extremely high ratio can adversely affect the conversion of reaction by reducing the concentration of H^+ ions in reaction mixture. Also, cost of methanol recovery in later stage increases. Hence, an adequate ratio should be used to maximize the recovery of FAME (Fig. 6(d)). As depicted in Fig. 6(d), the optimal methanol to fatty acids molar ratio was determined as 70 : 1.

3. Kinetics of Saponification of Lipids in Microwave Reactor

Reaction time affects conversion, and the conversion yield is increased by allowing more reaction time. The kinetics of saponification was studied at various temperatures and the results are depicted in Table 2. No significant yield was observed in the early stage of reaction (till 2 min), which might be due to low amount of lipids released. With further increase in reaction time, lipid release rates improved and a sharp rise in yield of soaps was observed. Longer reaction times facilitate sufficient interaction between lip-

Table 2. Kinetics of saponification reaction in microwave reactor at different temperatures (ethanol to dry biomass ratio=80 : 1, %KOH in ethanol=0.5, reaction time=7 min, microwave power=450 W)

Temperature (°C)	Rate constant K_{sapo}	n	Yield of soaps (%, w/w)						
			Time (min)						
			1	2	3	4	5	6	7
45	0.1703	0.85	8.56±0.19	21.25±0.32	35.56±0.15	39.68±0.29	48.95±0.35	51.14±0.25	52.23±0.18
50	0.186	0.997	10.18±0.14	35.85±0.09	51.89±0.18	60.74±0.16	62.18±0.28	65.22±0.27	65.25±0.31
55	0.2025	1.2	12.9±0.21	48.25±0.25	69.74±0.26	80.12±0.14	90.4±0.29	90.66±0.27	90.67±0.17
60	0.2255	1.129	15.19±0.17	55.19±0.19	75.25±0.29	95.14±0.30	97.24±0.24	98.22±0.26	98.25±0.14
65	0.2456	1.15	13.25±0.14	56.52±0.14	77.25±0.14	91.25±0.14	93.58±0.14	94.12±0.14	94.33±0.14

ids and KOH, leading to improved conversion of saponification reaction. Also,thermal effect caused by the microwaves increases the extractive properties of ethanol (diffusive extraction) and next, an extended microwave effect causes the penetration through the cell walls and forces out the lipids (from the biological matrix) into the solvent (disruptive extraction) [24]. Thus, microwaves reduce the reaction times enormously. In the present work, a reaction time of 7 min was sufficient to attain equilibrium at all temperatures.

The saponification reaction of lipids can be represented by,

$$\text{Lipids}+\text{KOH}\rightarrow\text{Soaps}+\text{Glycerin} \quad (5)$$

The rate constant of the reaction can be determined based on the increased amount of the product that occurs in some reaction time interval. In the present work, the increased amount of one product, i.e., soaps, was chosen. Therefore, the rate of reaction was expressed as,

$$\frac{d[C_{Soaps}]}{dt} = K_{sapo} C_{Soaps}^{n} \quad (6)$$

where K_{sapo} is rate constant of saponification reaction and C_{Soaps} represents concentration of soaps at any time 't'. Taking logarithms on both sides,

$$\ln\left[\frac{d[C_{Soaps}]}{dt}\right] = \ln K_{sapo} + n \ln C_{Soaps} \quad (7)$$

Hence, from the plot of $d[C_{Soaps}]/dt$ v/s C_{Soaps} the two constants n and K_{sapo} can be evaluated. Using the data in Table 2 and differential method, plots of ln $[d[C_{Soaps}]/dt]$ versus ln Y at different temperatures were made. A linear equation was obtained according to

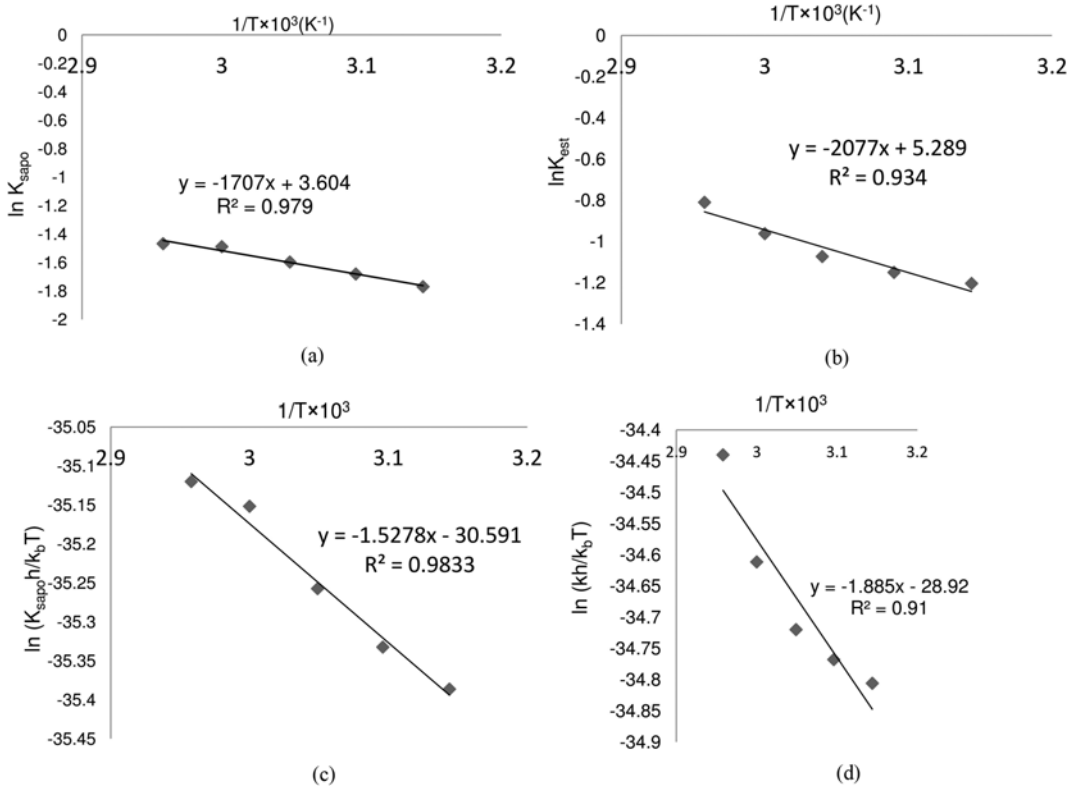

Fig. 7. (a) Arrhenius plot for saponification, (b) Arrhenius plot for esterification, (c) Eyring plot for Saponification, (d) Eyring plot for esterification.

Table 3. Kinetics of esterification of fatty acids (reaction conditions: Catalyst concentration=2.5%, molar ratio of methanol to lipid=70 : 1, microwave power=450 W)

Temperature (°C)	Rate constant K_{est}	Yield of soaps (%,w/w)							
		Time (min)							
		1	2	3	4	5	6	7	8
45	0.304	70.30±0.22	56.64±0.28	42.38±0.6	28.69±0.24	25.96±0.41	19.23±0.19	17.41±0.09	17.38±0.12
50	0.321	69.11±0.28	49.145±0.39	36.372±0.58	26.38±0.32	19.08±0.34	13.885±0.16	10.02±0.08	9.98±0.18
55	0.342	67.68±0.45	46.57±0.52	35.65±0.46	14.45±0.33	13.41±0.7	13.24±0.24	13.22±0.11	13.20±0.19
60	0.387	64.71±0.47	44.93±0.28	24.64±0.39	14.24±0.41	13.76±0.16	2.624±0.14	2.617±0.16	2.6±0.11
65	0.462	60.03±0.52	36.45±0.24	20.82±0.17	7.47±0.24	7.34±0.17	7.32±0.12	7.31±0.17	7.29±0.13

Table 4. Comparison of activation energy, Arrhenius factor and rate constant of esterification with literature

Source	Ea (kJ/mol)	A	K (min^{-1})	Reference
Palm fatty acid distillate	17.74	2.12	0.0299-0.0481	[22]
Jatropha	11.37	101.02	0.0026	[26]
Karanja	10.54	78.21	0.0025	[26]
Chlorella protothecoides	17.234	198.14	0.462	This study

Eq. (7). The order of the reaction was evaluated from the slopes of the straight lines, and the reaction rate constants were calculated from the intercept of the linear plot. The values of n ranged from 0.997 to 1.15. A fractional kinetic (with order of about 1.0652) was found. The reaction rate constant was found to increase with increasing temperature, and a maximum value of 0.2456 was attained at 65 °C. However, the yield was reduced to 94.33±0.14. The activation of energy of saponification and Arrhenius constant were determined as 14,177.76 J/mol and 36.74 by plotting ln K_{sapo} versus 1/T (Fig. 7(a)). According to our best knowledge, activation energy of saponification of lipids has not been reported in literature so far.

4. Kinetics of Esterification in Microwave Reactor

Acid-catalyzed esterification is a reversible reaction. However, given the significantly excess methanol used in the reaction as compared with fatty acids, the reverse reaction can be neglected. The experimental kinetic studies data could fit into a first-order reaction as [25],

$$-\frac{d[AV]}{dt} = k_{est} AV \tag{8}$$

where AV is the acid value of reaction mixture, k_{est} is the apparent is the apparent pseudo-first reaction rate constant (min^{-1}), which is expected to depend on the reaction temperature and the initial methanol and catalyst concentrations. Upon integration,

$$-\ln\frac{[AV]}{[AV_0]} = k_{est} \cdot t \tag{9}$$

where 't' is time the reaction time, AV_o represents the initial acid value of reaction mixture. k_{est} can be calculated from the slope of linear plot of Eq. (9). The kinetics of esterification was studied at various temperatures. The results are in Table 3. The rate constants increased with increasing temperature as expected. Maximum reduction in acid value was recorded at 60 °C (2.6 mg KOH/g reaction mixture) after 8 min. The activation energy was then determined by Arrhenius plot to be 17,234.52 J/mol (Fig. 7(b)). The value was compared with various studies as shown in Table 4. The rate constant obtained in the present study was nearly 12 times higher than values reported for palm acid fatty distillate and 185 times higher than for Jatropha and Karanja oils [26].

5. Thermodynamics of Saponification and Esterification

Gibb's free energy (ΔG) for the esterification and saponification can be calculated using the Eyring-Polanyi equation [27] as,

$$\Delta G = -RT \ln (kh/\kappa k_b T) \tag{10}$$

where k is the rate constant (min^{-1}), T is the absolute temperature (K), k_b is Boltzmann's constant (1.38×10^{-23} J/K), h is Planck's constant (6.63×10^{-34} J·s). κ, the prefactor is transmission coefficient, and this value is usually taken to be unity in most cases. Taking the natural logarithm on both sides of Eq. (10) and inserting the relation ΔG=ΔH− T ΔS in Eq. (5) gives,

$$\ln(kh/k_b T) = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \tag{11}$$

ΔH and ΔS for saponification and esterification were obtained through a linear plot of equation (ln (kh/k_bT) versus 1/T as shown in Fig. 7(c) and Fig. 7(d), respectively, and the values are depicted

Table 5. Comparison of thermodynamic properties of esterification of fatty acids with literature

Source	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/(mol·K)	Reference
Spirulina platensis	92.71	16.35	−232.83	[29]
Chlorella	96.128	36.124	−180.190	[30]
Chlorella protothecoides	95.591	15.671	−240.1	This Study

in Table 5.

The values of enthalpy of activation of saponification and esterification were 12.694 and 15.671 kJ/mol, indicating that energy input is required to raise the energy level and transform the reactants to their transition state. The values of entropy of activation (ΔS) were negative (−0.254 kJ/mol and −0.24 kJ/mol for saponification and esterification respectively), which may be a result of associative mechanism, which means that the reactant species combined with each other to form a transition state along the reaction pathway. Hence, the transition state has more ordered structure than the reactants in the ground state, resulting in a negative value of entropy of activation [28]. Gibb's free energy of activation was positive for both reactions (97.276 kJ/mol and 95.591 kJ/mol for saponification and esterification, respectively), which indicates the endergonic and unspontaneous nature of reaction. For a chemical reaction, the change in the Gibbs free energy function (ΔG) is the energy available to do work as the reaction proceeds from the given concentrations of reactant and products to chemical equilibrium. A positive value obtained in the present work indicates a higher energy level in the transition state than in the reactant species. As depicted in Table 5, thermodynamic properties obtained in present study slightly differed with those cited in literature [29,30], which may be due to difference in microalgae species used.

6. FAME Analysis

The total lipid and fatty acids content of *Chlorella protothecoides* biomass was 18.73 wt% and 12.82 wt% of dry biomass, respectively. The composition of final biodiesel is depicted in Table 6. The total FAME content in final biodiesel was 94.83%, which proves the excellent purity of biodiesel. The yield and purity of biodiesel in the present work were higher than the reports available in literature, who adopted a two-step procedure of saponification followed by esterification (Table 7). The greater purity obtained in the present work can be attributed to effective separation of unsaponification of matter. Out of total FAME constituents, saturated fatty acids (C14:0, C16:0, C18:0, C24:0) were 38.51, monosaturated fatty acids (C18:1, C22:1) were 39.11% and polyunsaturated fatty acids (C18:2, C18:3, C20:4 ω6) were 17.21%. The most abundant FAME was the oleic acid methyl ester, with a content of 38.04%. It is known that low amounts of polyunsaturated fatty acids and high oleic acid content improve oxidation stability, while high amounts of fully saturated fatty acids result in high cetane number and low iodine value. Also, the presence of double bonds favors the CFPP, avoiding the low-temperature formation of crystals which clog engine filters and nozzles. The oxidation stability of biodiesel was evaluated with a correlation developed by Knothe G [31] as,

$$\text{Oxidation stability index} = 3.91 - 0.045 \times \text{BAPE} \quad (12)$$

where BAPE is bis-allylic position equivalents in a sample which is calculated as,

$$\text{BAPE} = \text{wt\% of C18:2} + 2 \times \text{wt\% of C18:3} \quad (13)$$

OSI was evaluated as 3.171 h. According to EN 14214, oils having OSI greater than 3 h are considered relatively more stable and will not require addition of antioxidants for stabilization, i.e., biodiesel produced from such oils is expected to be utilized before OSI expires and degradation begins. Also, the maximum level of linolenic acid methyl esters (C18:3) must be 12%. The level was 1.14% in this study. The low ratio of the sums of unsaturated to saturated fatty acids (1.542) indicates the suitability of *Chlorella protothecoides* as a potential source for the production of biodiesel. Hence, theoretically, considering all these arguments, it can be predicted that the biodiesel obtained in the present study will meet the ASTM D 6751 specifications.

Table 6. Fatty acid composition of *Chlorella protothecoides*

Fatty acid	FAME composition (wt% in final biodiesel)
Myristic (14:0)	2.92
Palmitic (C16:0)	22.64
Stearic (C18:0)	7.19
Oleic (C18:1)	38.04
Linoleic (C18:2)	14.14
Linolenic (C18:3)	1.14
Arachidonic (C20:4 ω6)	1.93
Ocosanoic acid (C21:0)	2.54
Erucic acid (22:1)	1.07
Lignoceric acid (C_{24}:0)	3.22
Σ Total	94.83
Σ Saturated	38.51
Σ Monosaturated	39.11
Σ Polyunsaturated	17.21

CONCLUSIONS

A low-cost lab scale kitchen-modified microwave reactor with essential controls (Temperature, time, microwave power and stirring capacity) was used to conduct direct saponification of wet biomass of *Chlorella protothecoides* and esterification of fatty acids obtained through acidulation of soaps. Yield and purity of biodiesel obtained in our work (98.74% and 94.83%) were higher than the values reported in literature. The fatty acid profile of biodiesel indicated the presence of C14:0, C16:0, C18:0, C24:0, C18:1, C22:1, C18:2, C18:3 and C20:4 ω6. The most abundant FAME was the oleic acid methyl ester, with a content of 38.04%. Oxidation stability index

Table 7. Comparison of biodiesel yield and purity obtained in present work with literature

Microalgae species	Biodiesel yield (%)	Biodiesel purity	Reference
Nannochloropsis gaditana	80.9	96.5	[12]
Blend of *Nannochloropsis oculata*, *Isochrysis galbana*, *Pavlova lutheri*	32	77	[13]
Nannochloropsis gaditana	100	82.7	[15]
Chlorella protothecoides	98.74	94.83	Present study

(OSI) of final biodiesel was 3.171 h, which indicated its suitability to be a substitute for petroleum diesel.

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NOMENCLATURE

ΔG : standard free energy change
A : arrhenius constant
C_{soaps} : concentration of soaps
K_{sapo} : rate constant of saponification
n : order of saponification reaction
K_{est} : rate constant of esterification
h : Planck's constant
κ : transmission coefficient
ΔH : enthalpy of saponification and esterification
ΔS : standard entropy change
R : gas law constant

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