**Abstract:** This study describes the potential conversion of dried microalgae. *Chlorella vulgaris* (*C. vulgaris*) into fatty acid methyl ester (FAME) using the direct transesterification (DT) method and using ionic liquids (ILs) as a catalyst. In this work, the performance of monocationic IL, namely 1-butyl-1-methylpyrrolidinium bromide (IL 1), and dicationic IL, namely 1,4-bis(1-methylpyrrolidinium-1-yl)butane dibromide (IL 2), as catalysts was compared for DT of *C. vulgaris* under microwave irradiation. The results revealed that IL 2 showed a better performance in catalyzing the DT reaction by producing 87.9 mg/g% of FAME, while the use of IL 1 led to 74.3 mg/g% of FAME under optimum conditions. The kinetic study for direct transesterification of *C. vulgaris* showed that the reaction followed a first order kinetic reaction where the activation energies were calculated to be 22.2499 kJ mol$^{-1}$ and 22.0413 kJ mol$^{-1}$ for IL 1 and IL 2, respectively.

**Keywords:** ionic liquids; microalgae; biodiesel; catalysts

1. Introduction

Biodiesel production from microalgae is one of the most significant alternative energy sources. Microalgae offer the use of non-arable land for cultivation and grow rapidly throughout the year. Thus, it makes them a perfect substitute for liquid fossil fuels with respect to the cost, renewability and environmental concerns. In addition, microalgae have high lipid productivity, which is the main essential component for biodiesel conversion [1]. In 1942, Harder and von Witsch reported that microalgae could be a feasible source of lipids which are able to serve as food and can be converted to biofuel [2]. Since then, extensive research involving microalgae and their bioproducts has been explored. The conversion of microalgae into biodiesel typically includes the dehydration of algae, lipid extraction, transesterification reaction catalyzed by homogenous acid or base catalysts and a purification process. Thus, the complexity of the process makes biodiesel production from algae expensive [3,4]. Recently, a transesterification reaction has been performed using a biocatalyst such as lipase. However, there are some limitations in their use as a catalyst in transesterification, such as lower thermal stability of the biocatalyst, lower reaction rate and high enzyme cost [5]. Therefore, it is sensible to explore more economic and feasible approaches for biodiesel production in respect to process route and the type of catalyst.

The use of the microwave (MW) irradiation method has received great interest in biodiesel production from microalgae. This process is also known as direct transesteri-
fication (DT). DT of microalgae with the assistance of microwave is desirable to reduce processing time as well as to simplify the conversion steps and, consequently, reduce the overall cost for biodiesel production. DT is an alternative technique which facilitates the lipid conversion to alkyl esters directly, without extraction and drying process of the lipid. This method offers more energy-efficient and rapid heating in which molecules are directly heated through the interaction between microwave energy and molecular dipole moments of the starting materials [6–8]. MW energy penetrates deeply through the cell wall structure and generates a high temperature and pressure gradient, which facilitates cell wall degradation leading to higher lipid extraction. Hence, to further enhance the synergistic potential of microwave and IL in DT, two ILs were introduced in this process.

The conventional DT process typically utilizes volatile organic compounds that are harmful to the environment [9,10]. Hence, researchers started to explore green chemicals as a replacement, such as ionic liquids. Ionic liquids (ILs) are organic salts that consist of cations and anions with unique properties such as non-volatility and thermal stability and exhibit excellent physical characteristics that make them applicable in catalytic reactions. The use of ILs as a catalyst for biodiesel production offers an eco-friendly production route [11,12]. Ullah and co-workers reported that 3-methyl-1-(4-sulfo-butyl)-3H-imidazolium trifluoromethanesulfonate, (BSMIM)(CF$_3$SO$_3$) is a promising catalyst where it was able to catalyze transesterification reaction with high yield [13]. Furthermore, Wu et al. reported that hydrogen sulfate-based IL has converted up to 90% triglycerides for the transesterification of cottonseed oil to fatty acid methyl ester (FAME) [14].

In 2016, Wahidin and co-workers combined the benefits of the microwave method and ionic liquids (ILs) for DT of algal oils [15]. Two years after, they reported the synergistic effect of 1-ethyl-3-methylimidazolium methyl sulphate (EMIM)(MeSO$_4$) and MW using the DT technique, which exhibits 42% fatty acid methyl esters (FAME) per dry biomass weight [16]. Since limited research has been conducted so far on the use of IL-catalyzed DT of microalgae with the assistance of microwave, there is a lack of research findings and the data are still scarce. To date, most of the ILs used as catalysts in the DT reaction were imidazolium-based ILs, which are known to be expensive. In 2021, Malekghasemi and co-workers reported a good performance of phosphonium carboxylate ionic liquid, namely tetrabutylphosphonium formate, as a catalyst in the DT [17]. However, both imidazolium-based ILs and phosphonium-based ILs are found to be toxic [18]. In 2014, Neumann and co-workers reported the biodegradability properties of pyrrolidinium-based ILs [19]. Hence, considering both cost and toxicity properties, the pyrrolidinium-based ILs were chosen as the catalyst in this study. Besides that, the researchers also found that the anion and substituent on cation play a vital role in the acidic nature of ILs. Dicationic ILs possesses a higher melting point, wider liquid range and better thermal stability compared to conventional mono-cationic ILs [20,21]. Therefore, di-cationic ILs are potential candidates to be used as catalysts in this field. To the best of our knowledge, both mono and dicationic pyrrolidinium-based ILs have not been tested as catalysts in the DT of microalgae for FAME production. Hence, we wish to demonstrate the synergistic effect by combining microwave irradiation and the use of two types of pyrrolidinium-based ILs as catalysts for FAME production.

2. Results and Discussion

Owing to the biodegradability and unique properties of ILs, monocationic and dicationic pyrrolidinium-based ionic liquids, namely, 1-butyl-1-methylpyrrolidinium bromide and 1,4-bis(1-methylpyrrolidinium-1-yl) butane dibromide, were evaluated as catalysts in the FAME production under MW irradiation. The molecular structures of the synthesized ILs were confirmed using NMR and CHNS techniques. The structures of the synthesized monocationic (IL 1) and dicationic (IL 2) pyrrolidinium-based ILs are depicted in Figure 1. Meanwhile the NMR spectroscopy for both ILs were presented in Supplementary Materials (Figures S1–S4).
2.1. Optimization of Microalgae Conversion Using Pyrrolidinium-Based ILs: 1-Butyl-1-methylpyrrolidinium Bromide (IL 1) and 1,4-Bis(1-methylpyrrolidinium-1-yl) Butane Dibromide (IL 2)

Our previous work reported the FAME extraction from *C. vulgaris* using a conventional Bligh and Dyer method without the presence of IL as a catalyst, where 19.5 mg/g% was achieved. Additionally, the transesterification of *C. vulgaris* with the assistance of MW without the presence of IL was also evaluated, where a higher FAME yield of 26.8 mg/g% was achieved. The catalytic performance of pyrrolidinium based ILs was investigated for biodiesel production from dry microalgae, *C. vulgaris* powder by the direct transesterification reaction. Various factors such as catalyst loading, reaction time, reaction temperature and ratio of co-solvent, which may affect the transesterification reaction, were studied in detail.

To evaluate the performance of the catalyst, the amount of catalyst was varied in the range of 0.5 to 5.0 wt% relative to microalgae, while the other parameters were kept constant at 60 °C and 15 min reaction time with a ratio of methanol to hexane at 30:0. Results obtained during this present study have been compared with conventional Bligh and Dyer and MW methods. Figure 2 shows the FAME yield obtained in the reactions carried out with different catalyst loadings. It can be observed that the FAME yield increased with the increase in IL catalysts amount. The addition of catalyst molecules increased the opportunity for the triglycerides in the microalgae to be converted to FAME [22]. The highest yields of 62.0 mg/g% and 77.0 mg/g% were obtained when 3.5 wt% IL 1 and 3.0 wt% IL 2 were used for the transesterification of microalgae, respectively. The di-cationic IL 2 have a better performance in catalyzing the DT at lower concentration compared to monocationic IL 1. This could be due to the higher acidity of dicationic ILs as compared to monocationic ILs, as suggested by previous reports [23,24]. A further increase in the amount of IL catalysts did not enhance the yield for both cases. This can be explained by the increase in the viscosity of the reactants mixture, thus limiting the dispersion and the mass transfer of the immiscible reactants [25]. As the high dosage of IL catalyst also led to the additional cost [26], the catalyst loading of 3.5 wt% IL 1 and 3.0 wt% IL 2 was used for the following section.

![IL 1 and IL 2](image-url)  
**Figure 1.** Chemical structures of the monocationic (IL 1) and dicationic (IL 2) pyrrolidinium-based ILs.
Reaction time also plays a significant role in the transesterification of triglycerides and influences the yield of FAME. Figure 3 shows the effect of reaction time towards transesterification reaction, where the investigation was conducted by varying the reaction time from 5 to 30 min, keeping the other parameters constant at 60 °C, and the dosages of ILs catalyst were used at the optimum amount of 3.0 wt% and 3.5 wt% for IL 1 and IL 2, respectively. The experimental results showed that the FAME yield increased as the reaction time was prolonged for both ILs. The highest FAME yield was obtained at 20 min with 74.3 mg/g% and 87.9 mg/g% when IL 1 and IL 2 were used, respectively. However, a prolonged reaction time for both cases did not improve the performance. Hence, the optimum reaction time for both ILs was concluded to be 20 min.

Temperature is one of the important factors that highly affects the yield of FAME. In this study, the reaction temperature was varied from 50 °C to 80 °C, as shown in
Figure 4. Constant dosages of ILs catalyst were used at 3.0 wt% and 3.5 wt% for IL 1 and IL 2, correspondingly. The figure showed that at 50 °C, the FAME yield for the reactions catalyzed by IL 1 and IL 2 was relatively low, at only 53.1 mg/g% and 53.9 mg/g%, respectively. However, increasing the temperature increased the FAME yield due to the rise in the reaction rate [27]. For IL 2, no further increase in yield was observed when the temperature was higher than 60 °C.

Figure 4. Effect of temperature on FAME yield.

The effect of hexane as a co-solvent along with methanol in the DT of microalgae was investigated. Figure 5 depicts that the increase in the ratio of methanol to hexane reduced the FAME yield. The result is in contrast with the findings found in other literature [28]. This can be explained by the lower contact of lipid with methanol in the presence of hexane as co-solvent. Furthermore, the excess hexane led to lower heat transfer between lipid and methanol phase, and this phenomenon caused the reduction in FAME yield [29]. The MW irradiation method offers impressive synergy with the IL catalyst, especially those performed well on the polarization phenomenon and ionic conduction mechanism [30]. Figure 5 shows that, at a higher ratio of methanol to hexane (30:10 and 30:15), the FAME yield trend was transverse for the reactions catalyzed by IL 1 and IL 2. This can be explained by the difference in polarity of IL 1 and IL 2. A high amount of hexane in the reaction system may interrupt the behavior of IL 2, which leads to the less localized orientation of molecules under MW condition, thus reducing the FAME yield. Hence, this optimum condition was further used for the kinetic studies for both ILs.

2.2. FAME Analysis

The FAME composition extracted from C. vulgaris using ILs as a catalyst at optimum condition was determined using GC-FID, as shown in Table 1. Palmitic acid (C16:0) was found to be the dominant fatty acid in C. vulgaris, which was in agreement with a previous report [31]. The other major composition of FAME components found in C. vulgaris after transesterification using IL 1 and IL 2 was caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0) and cis-13,16-docosadienoic acid (C22:2). It was also observed that linoleic acid/linolelaidic acid (C18:2 C+T) was detected in GC analysis of the transesterification reaction using both ILs.
Figure 5. Effect of co-solvent on FAME yield.

Table 1. FAME components found C. vulgaris.

| Name of FAME                        | IL 1 (mg/g%) | IL 2 (mg/g%) |
|-------------------------------------|--------------|--------------|
| C6:0 Caproic acid                   | 14.64        | 17.68        |
| C8:0 Caprylic acid                  | 11.11        | 21.46        |
| C10:0 Capric acid                   | 10.03        | 7.66         |
| C11:0 Undecanoic acid               | 8.26         | 2.58         |
| C12:0 Lauric acid                   | 1.28         | 0.35         |
| C13:0 Tridecanoic acid              | ND           | 0.29         |
| C14:0 Myristic acid                 | ND           | 0.41         |
| C14:1 Mysteroleic acid              | ND           | ND           |
| C15:0 Pentadecanoic acid            | ND           | ND           |
| C15:1 cis,10 Pentadecanoic acid     | ND           | ND           |
| C16:0 Palmitic acid                 | 21.66        | 23.75        |
| C16:1 Palmitoleic acid              | 1.90         | 2.30         |
| C17:1 cis,10 Hepentadecanoic acid   | 2.37         | ND           |
| C18:0 Stearic acid                  | ND           | ND           |
| C18:1 C+T Elaidic acid + Oleic acid | ND           | 2.89         |
| C18:2 C+T Linoleic acid + Linolelaidic acid | 7.62 | 7.41 |
| C18:3n6 y-linolenic acid            | ND           | 2.99         |
| C18:3n3 Linolenic acid              | 2.17         | ND           |
| C20:0 Arachidic acid                | 3.22         | 2.85         |
| C20:2 cis-11,14-Eicosadienoic acid  | ND           | ND           |
| C22:2 cis-13,16-Docosadienoic acid  | 15.75        | 7.38         |

ND: not detected.

2.3. Kinetic Studies

In the excess of methanol, the equilibrium shifts to the right (forward reaction), which was assumed to be a single step process by neglecting the intermediate reaction [32,33]. The kinetic for this DT reaction was evaluated for the kinetic first order reaction. Based on Figure 6a,b, the plot for the DT reactions using both IL 1 and IL 2 showed a high correlation coefficient in the range of 0.9776 to 0.9975 for all temperatures. Thus, it showed that the DT follow the standard kinetic order for microalgae conversion to FAME.
Figure 6. First order reaction for the DT of microalgae at different temperature using IL 1 (a) and IL 2 (b) and the Arrhenius plot (c).
The use of IL 2 showed a faster reaction, where the rate constants were in the range of 0.003030 min$^{-1}$ to 0.004867 min$^{-1}$, as compared to the use of IL 1, where the rate constants were in the range of 0.002720 min$^{-1}$ to 0.004396 min$^{-1}$, as tabulated in Table 2. Figure 6c depicts the Arrhenius plot for the DT reactions using both IL 1 and IL 2. The figure shows that the correlation coefficients obtained for the DT using IL 1 and IL 2 were acceptable at $R^2 = 0.9107$ and $R^2 = 0.7930$, respectively. Hence, the graph was used to determine the kinetic parameters such as activation energy, $E_a$ and pre-exponential factor, $A$. Table 2 tabulates the value for both kinetic parameters where the use of IL 2 showed a lower activation energy (22.0413 kJ/mol) as compared to IL 1 ($E_a = 22.2499$ kJ/mol). Thus, lower energy was required to allow the direct transesterification of microalgae to happen. Hence, in this case, with lower IL2 amount (3.0 wt%), higher FAME yield (87.9 mg/g%) was achieved. The pre-exponential factor, representing the frequency of effective collision between molecules which leads to reaction, for the DT using IL 2, was also higher than the DT using IL 1. Hence, the use of IL 2 as a catalyst was more productive and accelerated the conversion of microalgae to FAME.

Table 2. Kinetic parameters of DT reaction for microalgae.

| Temperature (K) | k (min$^{-1}$) | $E_a$ (kJ/mol) | A       |
|----------------|---------------|----------------|---------|
| IL 1           |               |                |         |
| 323.15         | 0.002720      | 22.2499        | 11.2099 |
| 333.15         | 0.003970      |                |         |
| 343.15         | 0.004396      |                |         |
| IL 2           |               |                |         |
| 323.15         | 0.003030      | 22.0413        | 11.8580 |
| 333.15         | 0.004779      |                |         |
| 343.15         | 0.004867      |                |         |

This trend can be explained by the effect of ILs’ structure and the involvement of the functional group of the ILs in catalyzing the DT reactions, as shown in Figure 7. Generally, the DT process involved several steps. The reaction started with the attack of N+ of the pyrrolidinium cation of the IL on the carbonyl group of the ester, leading to the formation of carbocation. The reaction continued with the nucleophilic attack of the alcohol to produce the intermediate. It was followed by the removal of the first fatty acid methyl ester (FAME) and formation of diglycerides. The reaction continued with the attack of the IL cation towards diglycerides and monoglycerides to produce an overall of three FAME compounds and one glycerol. Figure 7b showed the general equation for direct transesterification of triglycerides in microalgae to form fatty acid methyl ester (FAME) and glycerol as the by-product. Various studies have described a similar mechanism for various catalysts [34,35]. Since the reaction was initiated by the cation, the higher amount of positively-charged atoms available in the cation would increase the speed of the reaction, as revealed by the finding of this study.
(a) Proposed mechanism for IL-catalyzed direct transesterification reaction

(b) General equation for transesterification of triglyceride to form FAME and glycerol

**Figure 7.** Proposed schematic representation of TG and methanol mechanism by pyrrolidinium-based IL as a catalyst, (a) the proposed mechanism for IL-catalyzed DT reaction, (b) the general equation for DT of TG to form FAME and glycerol as the by-product.
3. Materials and Methods

3.1. Materials

All solvents and chemical reagents were purchased from Merck, Darmstadt, Germany and used without any purification. The CAS number, source and grade of the chemicals are as follows: 1-bromobutane (109-65-9, Merck, 99%), 1-methylpyrrolidine (120-94-5, Merck, 98%), 1,4-dibromobutane (110-51-1, Merck, 99%), 2-propanol (67-63-0, Merck, 99.5%) and diethyl ether (60-29-7, Merck, 99.9%). Dried microalgae biomass *Chlorella vulgaris* (*C. vulgaris*) powder was purchased from a China Lover Store.

3.2. Preparations of Catalysts

Both ILs were synthesized using the procedure described by Burrel and co-workers [36]. The first, 1-butyl-1-methylpyrrolidinium bromide (IL 1), was synthesized by slowly adding an equimolar of 1-bromobutane (8.05 g, 0.059 mole) into a round bottom flask containing methylpyrrolidine (5 g, 0.059 mole). The reaction was conducted under solventless condition. The mixture was stirred at 150 rpm under reflux at 40 °C for 24 h. After the reaction, the mixture was left to cool at room temperature, resulting to yellowish mixture. Then, it was washed three times with 15 mL of diethyl ether to remove unreacted reactants. After that, it was washed three times with 15 mL of diethyl ether to remove unreacted reactants. After that, the resulting slightly yellowish thick liquid IL was dried in a vacuum oven for 24 h (89.9%).

\[ ^1H \text{ NMR (500 MHz, D}_2\text{O)}: \delta 3.40-3.37 (m, 4H), 3.23-3.19 (m, 2H), 2.92 (s, 3H), 2.09 (m, 4H), 1.69-1.62 (m, 2H), 1.31-1.23 (m, 2H), 0.83 (t, 3H, J = 7.5 Hz) (Figure S1). \]

\[ ^{13}C \text{ NMR (125 MHz, D}_2\text{O)}: \delta = 14.04, 19.78, 21.59, 25.46, 48.23, 63.42, 64.06 (Figure S2). \]

Theoretical calculation (%): C, 48.66; H, 9.07; N, 6.30. Experimental: C, 49.06; H, 9.17; N, 6.55.

For 1,4-bis(1-methylpyrrolidinium-1-yl) butane dibromide (IL 2), an equimolar of 1,4-dibromobutane (10.8 g, 0.05 mole) was slowly added into a round bottom flask containing methylpyrrolidine (4.3 g, 0.05 mole) in 2-propanol (50 mL) while being stirred at 150 rpm. The mixture was refluxed at 70 °C for 24 h. After the reaction, the mixture was left to cool at room temperature. Then, 2-propanol was removed using a rotary evaporator, resulting in a highly viscous dark brown mixture. After that, it was washed three times with 15 mL of diethyl ether before being dried under vacuum and yielding a solid product (86.0%).

\[ ^1H \text{ NMR (500 MHz, D}_2\text{O)}: \delta 3.58-3.38 (m, 12H), 3.08 (s, 6H), 2.24 (m, 8H), 1.98-1.96 (m, 4 H) (Figure S3). \]

\[ ^{13}C \text{ NMR (125 MHz, D}_2\text{O)}: \delta = 19.32, 21.72, 48.97, 63.37, 64.09 (Figure S4). \]

Theoretical calculation (%): C, 43.54; H, 7.83; N, 7.25. Experimental: C, 43.63; H, 8.31; N, 7.65.

3.3. Transesterification of Microalgae to Fatty Acid Methyl Ester (FAME)

3.3.1. Conventional Transesterification Reaction

For comparison purposes, the conventional Bligh and Dyer method was conducted without the presence of IL as a catalyst. First, 1 g of *C. vulgaris* was dissolved in a mixture of methanol, chloroform and water with a ratio of 1:2:0.8 (30 mL) for 2 h. Then, 10 mL of hexane was added and centrifuged at 4000 rpm for 15 min where two layers were formed. The top layer, which was the hexane layer, was separated, and hexane was removed completely using a rotary evaporator. The mass of lipid was weighed after the hexane had been removed. For the transesterification step, 1 mL of chloroform was added. Methanolysis was conducted at 60 °C for 3 h. The sample was further dried at 75 °C for 30 min and cooled to room temperature. Then, 0.3 mL of chloroform and 0.7 mL of heptane were added into the sample. The sample was then filtered and transferred into the vials. Finally, 1 mL of methyl heptadecanoate (C17:0) was added to the vial as a GC reference, and the sample was further analyzed by GC-FID.

3.3.2. Direct Transesterification Reaction

The direct transesterification (DT) reactions were conducted in a Milestone Flexi Wave microwave (MW) system. The experiments were conducted under microwave irradiation with exiting power of 700 W. To begin, 1 g of dried microalgae (*C. vulgaris*) was added into 30 mL of methanol/hexane solution containing the pre-determined amount of ionic
liquid in MW vessel. Then, the solution was exposed to microwave irradiation power. The biodiesel was then filtered through 0.22 µm nylon syringe filter for further FAME analysis. To note, in the case of a transesterification reaction involving 30:0 methanol to hexane ratio (which means no hexane was involved during the reaction), an extra step was added before the FAME analysis. N-hexane was used to separate the FAME from the residual.

3.3.3. FAME Analysis

The biodiesel samples obtained by the DT reaction were analyzed by an Agilent 7890A gas chromatograph system equipped with a J&W DB-WAX column (30 m × 0.25 mm, 0.25 µm thickness) with helium as the carrier gas. The flow rate of the carrier gas was set at 1.2 mL/min. One µL of the sample was injected, and the inlet was maintained at 250 °C. The oven was heated up from 50 °C to 220 °C for 10 min with 10 °C/min intervals. Then, the temperature was further increased to 250 °C for 10 min with 5 °C/min interval. The temperature of the injector and detector were set at 250 °C. Methyl heptadecanoate (C17:0) was used for internal standard for the quantification of the compounds. A Supelco 37-component mixture was used as the external standard to quantify the final product. Figures S5 and S6 present chromatogram of Supelco 37-component and the FAME sample (IL 1) at 60 °C, 20 min, in 30 mL of MeOH and 3.0% wt of IL). The mass and percentage of FAME yield were calculated using the following equations:

\[
m_{\text{FAME}} = \frac{A_{\text{FAME}} - A_{\text{IS}}}{A_{\text{IS}}} \times C_{\text{IS}} \times V_{\text{IS}}
\]

FAME yield (\(\text{mg/g} \)) = \(\frac{\text{weight of FAME obtained after DT (mg)}}{\text{weight of algae input (g)}} \times 100\%
\)

where \(A_{\text{FAME}}\) is the total peak area of FAME, \(A_{\text{IS}}\) is the peak area of the internal standard (methyl heptadecanoate), \(C_{\text{IS}}\) is the concentration (mg/L) of the internal standard and \(V_{\text{IS}}\) is the volume (mL) of the internal standard.

3.4. Kinetic Studies

The transesterification reaction involved the conversion of triglyceride (TG) in biodiesel feedstock, which, in this case, was microalgae to fatty acid methyl ester (FAME), which is the required biodiesel. Stoichiometrically, 3 moles of alcohol and 1 mole of TG are required to afford 3 moles of methyl ester (ME) and 1 mole of glycerol (GL) as the by-product. In general, this reaction consists of three consecutive reversible reactions, but with the excess use of methanol, the reaction is simplified as a single step forward reaction (Equation (3)).

\[
\text{TG} + 3\text{MeOH} \rightarrow 3\text{ME} + \text{GL}
\]

Thus, in this work, the transesterification reaction kinetics were calculated only for the forward reaction, following a first-order reaction, as a similar approach applied in other literature [26,36]. The rate of reaction is dependent only on the TG concentration, and the methanol concentration is assumed to be constant. The reverse reaction can be neglected, and the rate equation can be written as in Equation (4).

\[
-r = \frac{d[TG]}{dt} = k[TG]
\]

Generally, in the case of microalgae, the conversion of microalgae to FAME is used to monitor the kinetic of the reaction. Thus, the equation of conversion (Equation (5)) is considered. Rearranging the equation in terms of triglyceride concentration leads to Equation (6).

\[
x = \frac{[TG]_0 - [TG]}{[TG]_0}
\]

\[
[TG] = [TG]_0(1 - x)
\]
Thus, Equation (4) can be transformed into Equation (7). Integrating Equation (7) leads to Equation (8).

$$\frac{dx}{dt} = k[1 - x]$$  \hspace{0.5cm} (7)

$$- \ln(1 - x) = kt$$  \hspace{0.5cm} (8)

where $k$ is the rate constant, $x$ is the conversion of the microalgae to FAME, $[TG]_0$ is the initial concentration of triglyceride, which in this case is the microalgae, and $[TG]$ is the triglyceride (microalgae) concentration at time $t$. The data obtained from the conversion of triglycerides at different temperatures condition were used to find the rate constant $k$ by plotting the graph of $-\ln(1 - x)$ versus reaction time (Figure 6a,b). The rate constant was then used to obtain the activation energy by applying the Arrhenius equation (Equation (9)) to plot the Arrhenius graph (Figure 6c).

$$k = Ae^{\frac{-E_a}{RT}}$$  \hspace{0.5cm} (9)

where $k$ (min$^{-1}$) is the rate constant, $A$ is the pre-exponential factor, $R$ is the universal gas constant, $E_a$ is the activation energy and $T$ is the absolute temperature (K).

4. Conclusions

In the present study, we combined two environmentally benign methods, which were the use of microwave and ionic liquids (ILs), to assist the direct transesterification (DT) of microalgae to FAME. Monocationic and dicationic pyrrolidinium-based IL were synthesized and applied as a catalyst. The optimized conditions for the FAME production from microalgae were reactions conducted at 60 °C in 20 min with a catalyst dosage of 3.5 wt% and 3.0 wt% for IL 1 and IL 2 to produce 74.3 mg/g% and 87.9 mg/g% of FAME yields, respectively. The kinetic study demonstrated that the reaction followed a first order kinetic reaction with the activation energy, $E_a$ of 22.2499 kJ mol$^{-1}$ for DT using IL 1 and 22.0413 kJ mol$^{-1}$ for DT using IL 2. Thus, the use of IL 2 was favorable as compared to IL 1.

From this study, it can be concluded that the synergistic effect from ILs and microwave irradiation showed a beneficial method where only a small amount of ILs was required to act as a catalyst in the DT of microalgae to biodiesel.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/catal12030277/s1, Figure S1: $^1$H NMR of IL 1; Figure S2: $^{13}$C NMR of IL 1; Figure S3: $^1$H NMR of IL 2; Figure S4: $^{13}$C NMR of IL 2; Figure S5: Chromatogram of the 37 Component FAME mix obtained using GC-FID equipment with a DB-Wax column.; Figure S6: GC chromatogram for the characterization of the FAME sample (IL 1) at 60 °C, 20 min, in 30 mL of MeOH and 3.0% wt of IL.

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