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Biodiesel Production through Acid Catalyst In Situ Reactive Extraction of *Chlorella vulgaris* Foamate

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Abstract: A method of biodiesel production from the freshwater microalgae *Chlorella vulgaris* based on the conversion of the dewatered algal biomass from a foam column ("foamate") was investigated. The foam column collected and concentrated the microalgae. The foam was generated by passing air through a pool of algae, to which a collector/surfactant cetyltrimethylammonium bromide (CTAB) had been added. To produce biodiesel, the resultant "foamate" was esterified in situ using sulfuric acid and methanol. The effect of reaction temperature (30–70 °C), reaction time (30–120 min) and methanol/oil molar ratio (100–1000), were examined in a single-stage extraction–transesterification experiment on biodiesel yield at concentration of the catalyst H_2SO_4/oil molar ratio of (8.5/1). The thermodynamics and kinetics of transesterification of the microalgae oil were also investigated. The maximum biodiesel yield (96 ± 0.2%) was obtained at a reaction temperature of 70 °C, a reaction time of 90 min and methanol/oil molar ratio of 1000/1. Reaction kinetic parameters were determined that fitted the experimental data at all temperatures. A reversible reaction with first order forward and second order backward kinetics were found to be a good match for the experimental results. The kinetic model fitted experiments well under various temperatures and methanol/oil mole ratios. Under the most suitable conditions of reaction temperature, reaction time and methanol/oil molar ratio, the apparent activation energy was found to be 18.7 kJ/mol and pre-exponential factor 51.4 min^{-1}. The activation entropy (ΔS), change in Gibbs free energy (ΔG) and variation in activation enthalpy (ΔH) revealed that the transesterification reaction is endergonic and unspontaneous, while the endothermic nature of the reaction was confirmed by the positive value (16.6 kJ/mol) of the ΔH. The thermodynamic information and kinetic model reported here will provide valuable insight into the understanding of the in situ transesterification process from algae foamate to biodiesel.

Keywords: algal biofuels; foam columns; reactive extraction; in situ transesterification; thermodynamic parameters

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1. Introduction

Fatty acid esters are produced from vegetable oils and they have remarkably similar properties to fossil diesel fuels. The viscosity of the fuel is reduced by orders of magnitude through the transesterification reaction, a catalyzed process whereby the vegetable oil reacts with short-chain alcohols, to produce shorter chain linear alkyl esters. The exact quality of the biodiesel generated from the transesterification reaction is a strong function of the source feedstock, as it is a function of the degree and length of saturation of the fatty acid chains. Biodiesel is a non-toxic, biodegradable renewable fuel [1]. Biodiesel production using feedstocks from plant-derived oils such as sunflower oil, soybean oil, rapeseed oil, palm oil, coconut oil, as well as animal oils and greases, has been widely investigated and is a widespread commercial process. However, the competitiveness of biodiesel with petro-diesel is constrained by the cost of vegetable oils, which affects its

final selling price [2]. The high price of biodiesel production, which is mostly related to the price of feedstock processing, has limited its widespread use in many countries [3]. However, this challenge has been addressed in the USA and EU amongst others by policy instruments such as subsidies and minimum content legislation and consequently, millions of tons of biodiesel are produced every year [4].

The use of vegetable oil for biodiesel may adversely affect food supplies. These constraints on biodiesel production can be resolved by using non-edible feedstocks such as jatropha oil, castor oil, rubber seed oil and algae oil that will not compete with food [5]. Compared to other non-edible plant-derived oil, the use of microalgae oil for biodiesel production has several benefits. The process of microalgae cultivation for biodiesel production is sustainable and microalgae possess a robust photosynthetic ability and a rapid growth rate. Microalgae cultivation offers higher productivity than other plant-derived sources per unit land area. The extracted oil from microalgae has superior lipid quality compared to vegetable-derived oil [6] because the suitability of microalgal biomass as a biofuel feedstock is closely related its saturated and monounsaturated fatty acids content. The high proportion of saturated and monounsaturated fatty acids in this alga is beneficial from a fuel quality standpoint in that fuel polymerization during combustion would be substantially lower than for polyunsaturated fatty acid-derived fuel. Furthermore, generally, biodiesel produced from algal lipids is nonhazardous and highly eco-friendly [7]. A further advantage is that, if the algae are cultivated utilizing power plant flue gas or perhaps other sources of sequestered CO_2, biodiesel generation from algae has the added benefit of lowering nitrogen oxide and carbon dioxide emissions from power plants [8]. Recent studies on the production of biodiesel have focused on using an in situ reactive extraction of microalgae. For high viscosity oils, the most viable solution is the transesterification of algae oil to its related fatty ester ("biodiesel"). Singh and Patidar [9], Xia et al. [10] and Laamanen et al. [11] have established that foam flotation as a microalgae harvesting technique offers better harvesting characteristics compared to other techniques and has significant added value beyond energy-efficient biomass recovery. Research efforts have concentrated on improving the techniques of microalgae harvesting, methods of oil extraction and the reaction conditions. An extensive review by Bilad et al. [12] found that membrane technology has been employed as a viable means of cultivating and harvesting of microalgae. For harvesting microalgae biomass, Coward et al. [13] reported the potential of employing a foam column. The foam column combines dispersed air floatation with foam fractionation and the process offers the advantage of being more cost-effective compared to commonly used bulk harvesting technologies. Microalgae biomass recovery technology has been reported to significantly influence the overall economy of microalgae production [14]. Alkarawi et al. [15] investigated the use of continuous harvesting of microalgae biomass using a foam floatation column. The study revealed that foam floatation has the potential to be used for a continuous bulk harvesting of microalgae biomass that could either be utilized for biodiesel production or other high-value chemical products. Acid-catalyzed transesterification, among the various biodiesel manufacturing techniques, allows for utilization of feedstocks with relatively high free fatty acid concentration. Many of the cheaper feedstocks, such as yellow and brown grease, have very high fatty acid contents and cannot be transformed into biodiesel using the conventional alkaline catalyst transesterification. Furthermore, because soap does not form when acid is used as a catalyst, downstream separation and purification operations are simplified [16]. Microalgae-derived biodiesel is a more renewable, environmentally sustainable form of diesel [17,18]. However, the high costs involved in the production of microalgae are a drawback of this technology [19]. To address this high cost, cost-effective processing methods must be developed.

Product recovery yields can be improved by cell disruption. Methods include chemical and mechanical techniques, such as sonication, microwave radiation, enzymatic and surfactant [20,21]. These techniques' efficiencies are influenced by microalgae species, cell wall composition, culture age [22] and cell wall thickness and cell size [23]. None of these technologies is currently proven at large scale. Cell disruption strategies utilised

to produce microalgae-derived biodiesel must be scalable and cost-effective. Given these considerations, some disruption techniques are less suitable than others due to their high energy requirements or poor scalability [24,25]. Surfactant treatment, on the other hand, has been shown to be successful in disrupting microalgae cells [25,26] and is suited to large-scale operation, with food-grade surfactants such as cetyltrimethylammonium bromide (CTAB) available [27]. A mechanism postulated for CTAB-induced surfactant cell breakdown is based upon electrostatic and hydrophobic interactions [28]. The negatively charged microalgae cell surface electrostatically binds to the positively charged cationic surfactant head groups contained in CTAB [28,29] in the first phase of the adsorption procedure [25]. Hydrophobic elements of the cytoplasmic membrane subsequently interact with the hydrophobic tail ends of the surfactant, creating micelles that disrupt the extracellular environment [29–32].

It has been shown that the amounts of lipid retrieved from microalgae harvested by foam flotation with the surfactant cetyl trimethyl ammonium bromide (CTAB) were much greater than those recovered from centrifuged cells [33]. Coward et al. [33] reported that cells extracted using CTAB-assisted foam flotation also had a lipid composition that was better suited to biodiesel conversion, with a higher concentration of saturated fatty acids. However, it has also been reported that CTAB-aided in situ cell lysis occurs by solubilizing the phospholipid bilayer, hence increasing the quantity of extractable lipid [34].

Besides improving microalgae harvesting, it is important also to find optimal operating conditions for the biodiesel production reaction. Parameters such as alcohol-to-oil molar ratio, reaction temperature, catalyst concentration, type of catalysts and reaction time have been proven to influence biodiesel yield. Methanol has traditionally been utilized in the production of biodiesel via the transesterification reaction since it is the lowest cost alcohol [7]. An extensive review by Verma and Sharma [35] investigated the effect of parameters such as catalyst type/concentration, alcohol/oil molar ratio, stirring speed, reaction temperature and reaction time on biodiesel yield. The study revealed that reaction temperature, reaction time and the alcohol/oil molar ratio were the most significant parameters. Specifically, the type of catalyst used in the transesterification reactions significantly influences the end products and the overall process. Both heterogeneous catalysts such as CaO, MgO and ZnO and homogeneous catalysts such as KOH and NaOH have been extensively investigated for the transesterification reaction and biodiesel production. Acid-catalyzed transesterification, among the various biodiesel manufacturing techniques, allows for the utilization of feedstock with a greater free fatty acid concentration and water tolerance. Moreover, using homogeneous catalysts requires a shorter reaction time, albeit it at the expense of not being able to reuse the catalyst.

Although the transesterification of oilseed and microalgae into biodiesel using reactive extraction with homogeneous and heterogeneous catalysts has been investigated before, the application of foam columns in dewatering and disruption of microalgae cells that are used as feedstock to reactive extraction to produce biodiesel is yet to be investigated. This study focuses on the production of biodiesel from *Chlorella vulgaris* "foamate", the top product stream from a foam column, using reactive extraction (sometimes referred to as in situ transesterification). In this study, a continuous foam column was employed in which foam drainage was enhanced by the use of a riser with successive contraction and expansion, as first proposed and investigated by Li et al. [36] and Li et al. [37].

The effect of exposing concentrated disrupted algal biomass to fast reactive extraction to attain a high FAME yield in a minimal reaction time was investigated. Process parameters such as reaction time, reaction temperature and methanol-to-oil molar ratio were investigated and the results used to investigate the kinetics and derive thermodynamic quantities (ΔH and ΔS) for the single-stage extraction–transesterification process of biodiesel production from *Chlorella vulgaris* algae.

2. Materials and Method

2.1. Materials

All experiments were performed using freshwater *C. vulgaris* which was grown under non-sterile conditions using BG-11 medium in 20-L polycarbonate carboys (Nalgene) at 22 °C. Cold and warm fluorescent lights (average illuminance of 2400 lux) were used, with a light regime of 16L:8D. Culture agitation and gas transfer were conducted using an aquarium air pump. Surface material (cetyltrimethylammonium bromide) CTAB, $CH_3(CH_2)_{15}N(Br)(CH_3)_3$; Sigma-Aldrich, Gillingham, UK. Methanol (anhydrous, (99.8%) was obtained from Sigma-Aldrich, Gillingham, UK), n-Hexane (96%, Merck), and chloroform were utilized as solvents in this study (99%, was supplied by Fisher Scientific, Loughborough, UK). The internal reference for FAMEs measurement was methyl heptadecanoate C17:0 (99 percent, Sigma-Aldrich, Gillingham, UK).

2.2. Experimental Procedure

The *Chlorella vulgaris* foamate that was used as the feedstock for these experiments is the top product stream from a foam column with a height of 135 cm and a diameter of 5 cm with a contraction expansion section of 15 cm height and diameter variation ratio of 0.2. It was used to dewater the *Chlorella vulgaris* culture under operating conditions of 1 L/min air flow rate, 0.2 L/min feed flow rate and 35 mg/L surfactant CTAB in an algal culture containing 5×10^6 cell/mL. Figure 1 shows the foam-assisted dispersed air flotation apparatus used for microalgae harvesting, which at the same time concentrated any CTAB not adsorbed on the surface of the cells in the foamate. The foamate, which consisted of harvested microalgae with biomass concentration (261 mg/mL), culture medium and CTAB, was collected from the foam collector. To ensure that enough biomass was available for the in situ reactive extraction studies, a total of 30 harvesting runs were performed, and the total volume of culture that was harvested was 2 L. To guarantee uniformity of the samples prior to the reactive extraction tests, a paste was formed by thawing the microalgal pellets from all the collected runs at room temperature and then blending them (Fisher Scientific, Ottawa, ON, Canada, Thermix Stirring Hot Plate, Model 310T). The biomass foamate moisture content was determined using Karl Fischer titration (as described in Section 2.3.1) once the samples were combined. The lipid content of the microalgae was determined using the methods described in Section 2.3.2. The microalgae were then divided into tubes, with each containing 800 mg. For easy identification, each of the tubes was labeled, capped and covered with parafilm for the in situ reactive extraction studies.

A total of 48 experimental runs were set up to investigate the effect of reaction time, methanol/oil molar ratio and reaction temperature on the transesterification of the foamate samples catalyzed by sulphuric acid. The fixed variables include the mass of algae biomass (800 mg), mixing rate (380 rpm) and the concentration of the catalyst H_2SO_4 was 8.5:1 sulfuric acid to oil molar ratio. The methanol to oil molar ratios were 100:1, 400:1, 700:1 and 1000:1; the reaction temperatures were 30 °C, 50 °C and 70 °C; and the reaction times were 30, 60, 90 and 120 min. The percentage yield of fatty acid methyl ester (FAME) achieved was the response variable in the experimental work.

In situ transesterification was performed in 20 mL test tubes. Prior to initiating the transesterification reaction, the H_2SO_4 was used at an 8.5:1 (acid/oil) mole ratio and dissolved in methanol, then the solution was heated for 5 min to the reaction temperature before being added to 800 mg of the foam algae in the tube. The mixture in each of the tubes was placed in an incubator (IKA KS 4000 iControl) and continuously stirred at 380 rpm at constant temperature. After the stipulated time for the transesterification, the reaction was terminated by adding sodium bicarbonate to each of the tubes. The tubes were allowed to cool in an ice bath and subsequently, all the contents were removed and filtered to separate the remaining solid residues of algae. The filtered mixture was centrifuged to split the biodiesel phase from glycerol using a "SCI QUIP sigma 3–16p" centrifuge for 20 min at $4000\times$ g before being transferred to a separation funnel. FAME and methanol were abundant in the higher layer, while excess methanol, glycerol and other polar chemicals

were abundant in the lower layer. The topmost layer was separated and warm water was used to wash the final product methyl esters layer to remove the catalyst, excess alcohol and glycerol. The washing was carried out until all the catalyst from the biodiesel layer had been removed. The resultant product combination was boiled to remove excess alcohol and water before being collected in a bottle for further chemical analysis to determine the composition of the FAME.

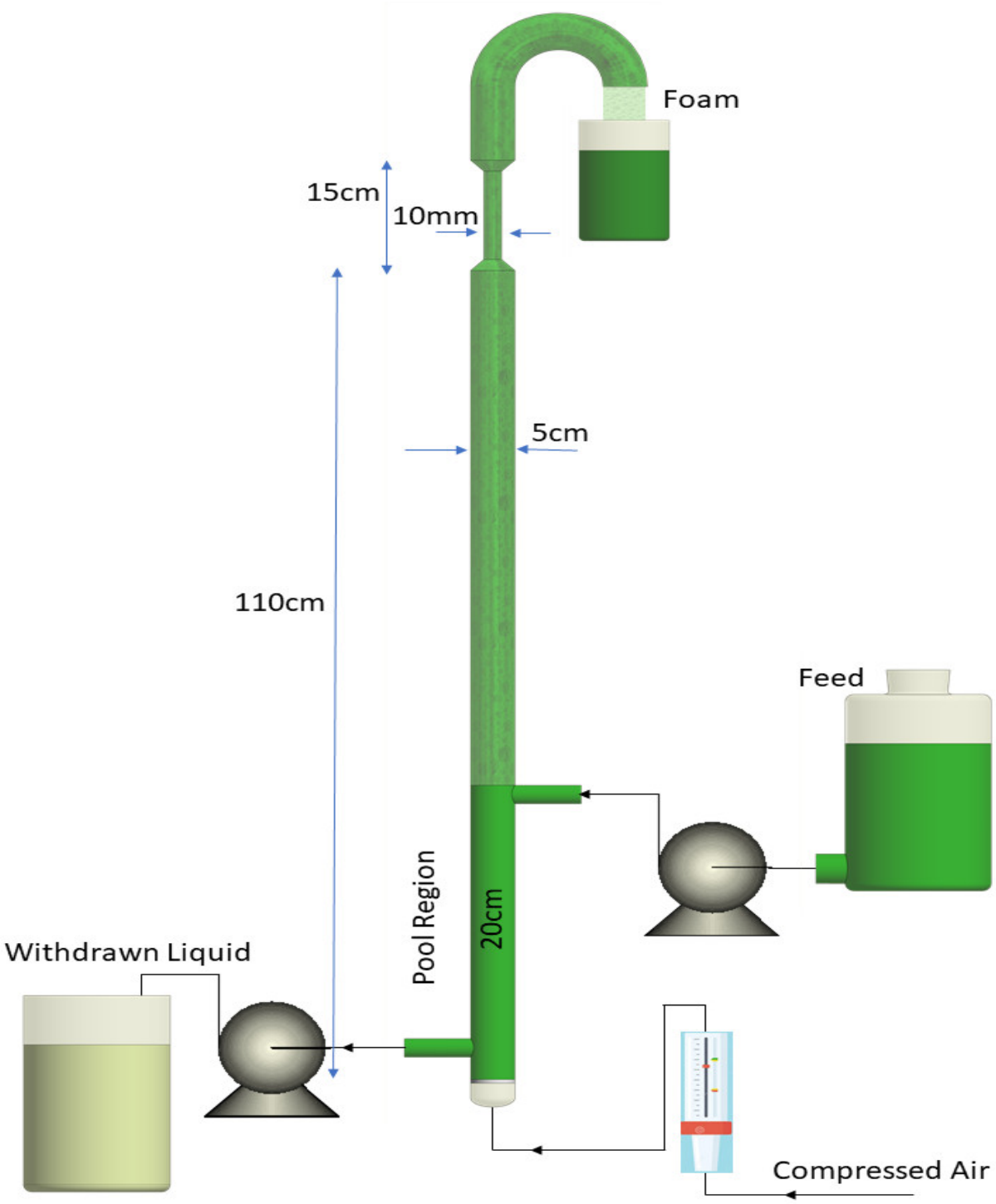
2.3. Analytical Methods

2.3.1. Moisture Content Measurement

Moisture contents of the algae biomass foam samples were measured by Karl Fischer titration. Karl Fischer titration was used as a specific standard method for the determination of water content in the liquid surrounding the cells. HPLC grade methanol (Merck, Gillingham, UK) was used to determine algae moisture content. Thus, 300 mg algae biomass was shaken in 7 mL of HPLC grade methanol (Merck) for 1 h at room temperature. The sample was filtered, and the moisture content of the filtrate was determined by Karl Fischer titration using a Metrohm 787 volumetric titrator (Metrohm USA, Riverview, FL, USA) with methanol as solvent and HYDRANAL Karl Fischer Composite 5 (Fluka, Sigma-Aldrich Schweiz, Buchs, Switzerland) as a titrant. Moisture contents reported have been adjusted for the inherent water content of the wash methanol.

(**a**)

Figure 1. *Cont.*



(**b**)

Figure 1. System to prepare chlorella vulgaris algae for reactive extraction. (**a**) photo of dispersed air flotation apparatus with foam assistance(rising) and (**b**) sketch of flotation rig.

2.3.2. *Chlorella vulgaris* Microalgae Total Maximum Fames and Total Lipid

A modified Bligh and Dyer method [38] was used to measure the total lipid content of *Chlorella vulgaris* microalgae capable of being converted to FAMEs. This approach was used to determine the maximum possible conversion of the fatty acids to biodiesel. Foamate of *Chlorella vulgaris* microalgae was dried in an oven for 6 h at 60 °C. It was then put into a

flask. 0.5 g of dry microalgae was mixed with 15 mL mixture of methanol and chloroform (2:1 v/v) and was centrifuged for 20 min. The sample was agitated using a magnetic stirrer at 180 rpm and 60 °C for 2 h to extract the lipids. Next, the sample was centrifuged for 10 min at 5000 rpm, and the supernatant collected and saved. To ensure the extraction of all the lipids, this was performed twice more on the leftover biomass. The solutions collected were then combined, and the methanol and chloroform evaporated using a vacuum oven (Lab Companion OV-11/12, JEIO TECH | Apex Scientific South Africa) at vacuum pressure 0.1 MPa. Residual lipids in the flask were weighed. To determine the total lipids capable of converting to FAMEs, extracted lipids were first reacted with 2 mL methanol containing 2% (w/v) KOH for 15 min at 65 °C; subsequently, 2 mL methanol containing 5% (v/v) H_2SO_4 was added to the solution and the flask was heated for another 15 min at 65 °C. The FAMEs were then extracted using n-hexane. As an internal standard, 10 µL of methyl heptadecanoate solution at a concentration of 50 mg/mL was added to the sample. Gas chromatography, described in the next section, was used to determine the lipid content.

2.3.3. Determination of Total Mass of Methyl Ester by Gas Chromatography with Flame Ionization Detector

The FAME concentration following in situ transesterification was determined using the European standard procedure [39]. A gas chromatograph (GC HEWLETT PACKARD 5890SERIES II) was used to measure the total FAME. The GC configuration consisted of an Elite-5MS capillary column with a head pressure of 4.5 psi and a carrier gas of H_e (99.9%) at a flow rate of 1 mL/min, in splitless mode. The GC was operated at a hydrogen pressure of 32 psi, air pressure of 7 psi and H_e pressure, of 7 psi. The temperature program of the GC was as follows: Start at 120 °C, hold for 2 min then increase the temperature to 260 °C at 7 °C/min. The temperature of the injector and detector were both fixed at 250 °C. The samples were mixed with 1 mL of an internal reference methyl heptadecanoate solution (C17:0 methyl ester in iso-propanol) with a concentration of 10 gm/L in 2 mL vials to measure FAME concentration. The measurement data was obtained using Data Apex Clarity software, UK, after 1 µL of the combination was injected into the GC. A series of pure FAME compounds (C16:0, C17:0, C20:0 and C22:1) and a grain FAME mix (Sigma Aldrich, Gillingham, UK, 10 mg/mL) were examined at the same GC conditions as the FAME samples to distinguish the chemicals in the FAME chromatogram. In order to ensure accuracy, the analyses of the samples using the GC-FID were performed twice.

The yield of FAME was determined using Equation (1)

$$\text{FAME Yield } \% \left(\frac{\text{wt}}{\text{wt}}\right) = \frac{\left(\frac{(\text{Weight of FAMEs})}{\text{weight of algae}}\right)_{\text{S.C}}}{\left(\frac{(\text{max weight of FAMEs available in the algae})}{\text{weight of algae}}\right)_{\text{B.D}}} \times 100\% \quad (1)$$

where the single-stage transesterification condition and Bligh & Dyer procedure, respectively, are denoted by S.C. and B.D.

3. Results and Discussion

The moisture content of the algae using Karl Fischer titration revealed that the algae foamate consisted of 37.0 wt.% dry mass. This value was used to determine the moisture content of microalgae. The separation of the microalgae cells from the culture medium was achieved using foam flotation with the aid of a cationic surfactant, cetyltrimethylammonium bromide (CTAB). In addition to its significant role in dewatering, CTAB can disrupt the walls of algae and improve lipid recovery. Figure 2 shows the microalgae cells before and after treatment with a foam column. The microalgae are distinct, individual cells before the treatment (Figure 2a), but afterwards (Figure 2b) the cell walls have been disrupted, releasing lipid, and the cells are more aggregated. Cell debris can be seen within the image as small green clumps filling the space between cells. Physical deformation of the microalgae cells was observed under the microscope to continue after treatment with

the foam column. Treatment of microalgae with CTAB has previously been observed to significantly enhance cell disruption [40].

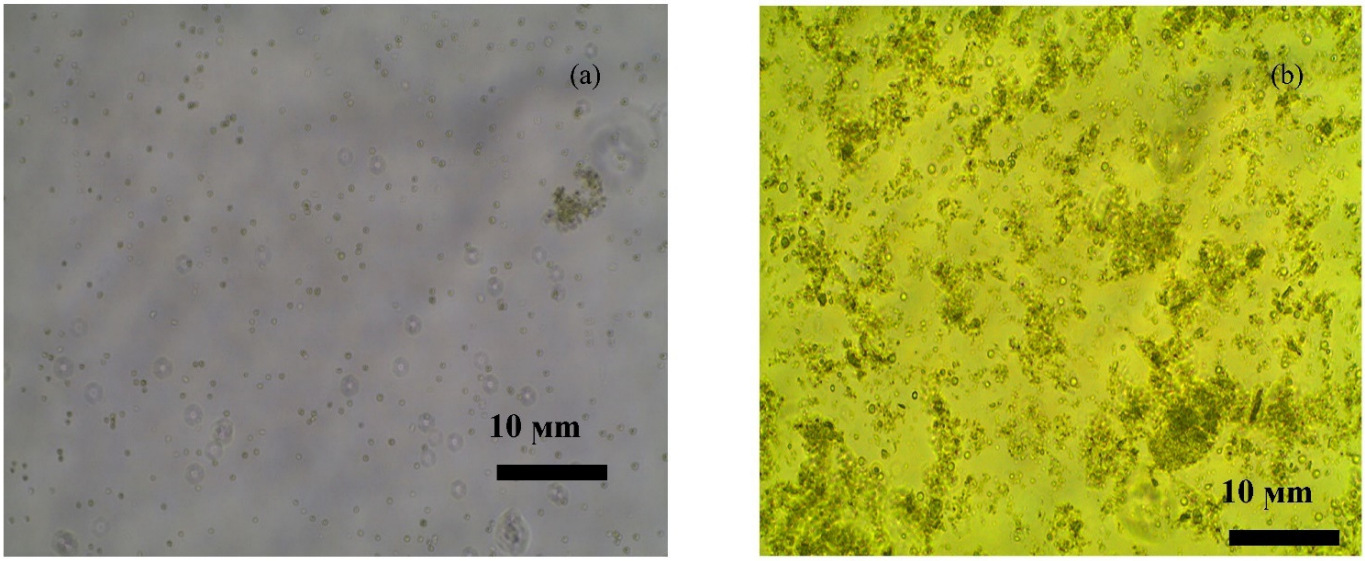


Figure 2. Microscope image of the freshwater microalgae species, Chlorella vulgaris at ×40 magnification (**a**) before and (**b**) after treatment with a foam column.

3.1. Process Parameters

3.1.1. Methanol/Oil Mole Ratio and Reaction Temperature

Figure 3 shows the effect of methanol/oil mole ratio on reaction temperatures in the range of 30–70 °C on the yield of the FAME. Figure 3a shows the effect of varying the methanol/oil mole ratio and reaction temperature at a fixed reaction time of 30 min on the yield of the FAME. Figure 3b–d shows the effect of varying the reaction time.

Increasing the methanol/oil ratio resulted in increased biodiesel yield for the reaction temperature at 30 °C, 50 °C and 70 °C. However, at 1000:1 methanol/oil mole ratio it can be seen that the biodiesel yield increases from 32% at 30 °C to 53% at 70 °C. Clearly, both the methanol/oil mole ratio and the transesterification reaction temperature significantly influence the biodiesel yield. Similarly, as seen in Figure 3b, increasing the reaction time to 60 min leads to an increase in the biodiesel yields with an increase in the reaction temperature and the methanol/oil mole ratio. The biodiesel yield increased from 64% at 3 °C to 88% at 70 °C. As shown in Figure 3c,d, a further increase in the reaction time to 90 and 120 min does not significantly increase the biodiesel yield as the reaction temperature and the methanol/oil mole ratio increase. The trend obtained for the effect of methanol/oil mole ratio on the biodiesel yield produced from microalgae in this study is consistent with that reported by Hasni, Ilham, Dharma and Varman [41] for biodiesel production from *Brucea javanica* seed oil. Baskar, Gurugulladevi, Nishanthini, Aiswarya and Tamilarasan [42] investigated the effect of oil/methanol ratio on biodiesel yield produced from mahua oil using manganese doped zinc oxide nano catalyst. The results showed that a maximum biodiesel yield of 96% was obtained at an oil/methanol ratio of 1.07% (*v*/*v*). Nautiyal, Subramanian and Dastidar [43] produced biodiesel from *Spirulina platensis* algae biomass by a single-stage extraction–transesterification. They studied the effect of algae biomass to methanol ratio (*w*/*v*) and achieved a maximum biodiesel yield of (75%) at 1/4 algae biomass to methanol ratio.

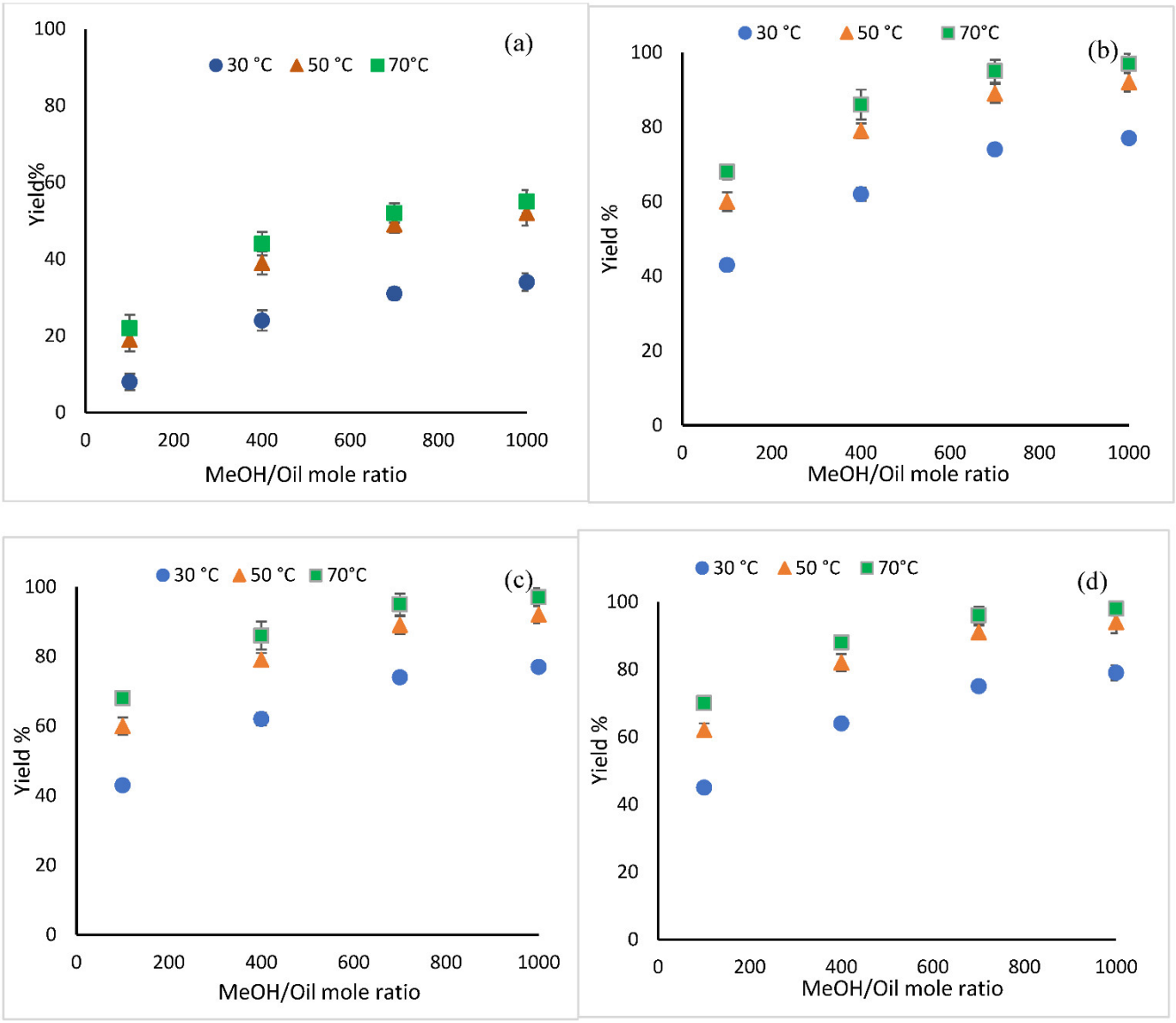


Figure 3. Effect of MeOH/Oil Molar ratio at varying reaction temperature on the FAME yield at reaction times of (**a**) 30 min (**b**) 60 min (**c**) 90 min and (**d**) 120 min.

Previous research [44] into in situ and conventional techniques for converting TAG to FAME found that a rise in the reaction temperature reduced the time required to reach a maximum yield of FAME, as would be expected assuming Arrhenius kinetics. Tests were conducted to determine the minimum temperature needed to achieve the maximum yield of FAME in short reaction times ranging from 30 to 120 min without needing temperatures substantially greater than the boiling point of methanol. The increasing rate of reaction observed here with rising temperature agrees with other articles [45–48] on acid-catalyzed transesterification reactions performed directly on biomass or extracted oil.

3.1.2. Reaction Time

The effect of reaction time on biodiesel yield during transesterification reaction using methanol/oil ratios of 100, 400, 700 and 1000 is depicted in Figure 4. In Figure 4a, at a reaction temperature of 30 °C, an increase in the reaction time resulted in a corresponding increase in biodiesel yields. The maximum biodiesel yields of 43%, 63%, 74% and 77% were obtained using a methanol/oil ratio of 100, 400, 700 and 1000 respectively. A similar trend is also observed at a reaction temperature of 50 °C, as depicted in Figure 4b. At methanol/oil

ratios of 100, 400, 700 and 1000, the maximum biodiesel yields were 60%, 79%, 89% and 92%, respectively. The results agree with the previous work [49–51] on the acid-catalyzed in situ transesterifications of algae biomass for biodiesel production.

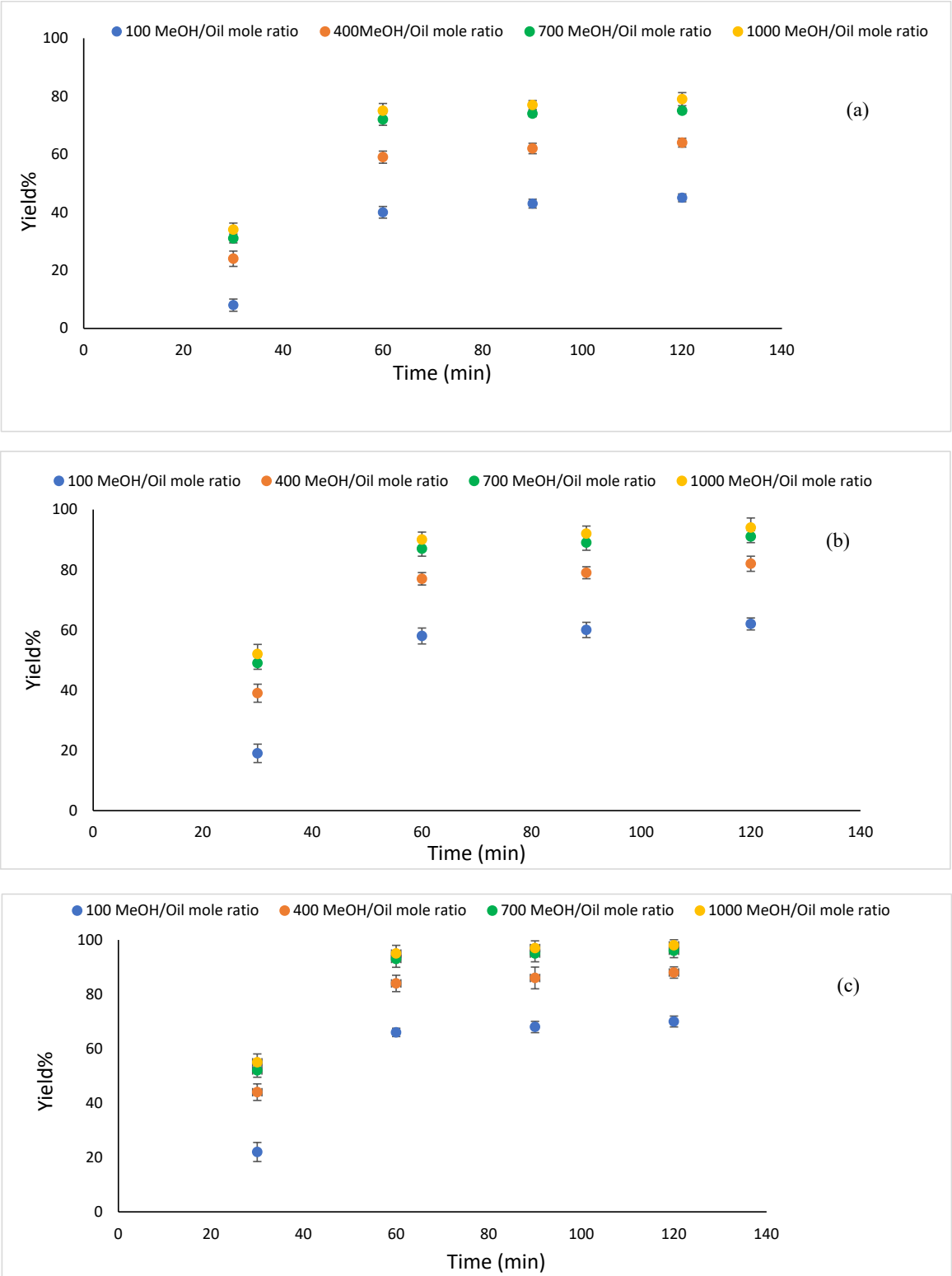


Figure 4. Effect of reaction time on the FAME yield at reaction temperature of (**a**) 30 °C (**b**) 50 °C and (**c**) 70 °C.

A comparison of the biodiesel yields obtained in this study with those previously reported in the reactive extraction literature using microalgal biomass feedstocks is presented in Table 1. It can be seen that the highest biodiesel yield of 96% obtained from the H_2SO_4-catalyzed transesterification of the in-situ transesterification of microalgae is comparable with those reported in the literature. Higher biodiesel yields of 96%, and 97%, have been reported for transesterification of *Chlorella vulgaris* to biodiesel using H_2SO_4 catalyst.

Table 1. Comparison of the present study with literature.

Feedstock	Temperature (°C)	Solvent	Catalyst (Oil Basis) (mol/mol)	Molar Ratio (Solvent: Oil)	Reaction Time (min)	Max. Yield (Oil Basis) (%)	References
Chlorella vulgaris	70	MeOH	H_2SO_4 0.85:1	1000:1	90	96	This study
Chlorella vulgaris	60	MeOH	H_2SO_4 650:1	10,000:1	120	ca.96	[52]
Chloroparva pannonica	25	MeOH	H_2SO_4 0.70:1	830:1	15	ca.97	[53]
Dry *Chlorella vulgaris*	50	MeOH	H_2SO_4 0.336:1	800:1	60	ca. 90	[54]
Chlorella vulgaris	60	MeOH	H_2SO_4 0.35:1	600:1	1200	97	[49]

Table 1 shows that increasing the amount of acid catalyst and reaction time increases the yield when high solvent concentrations are used. Increasing the acid concentration by a factor of 1857 has a similar impact to increasing the reaction time by 10. A high FAME yield was observed at H_2SO_4/oil mole ratio of 0.35:1 and 1200 min. When compared to a higher H_2SO_4 concentration of 650:1120 min of residence time achieved maximum FAME content.

3.2. Kinetic Study of the Transesterification Process

In order to determine the kinetics of the transesterification of microalgae oil to biodiesel, the effects of reaction time and temperature were investigated as depicted in Figure 4. The transesterification reaction can be represented by Equations (2)–(4).

$$\mathrm{TG} + \mathrm{MeOH} \underset{k_2}{\overset{k_1}{\Leftrightarrow}} \mathrm{DG} + \mathrm{FAME} \quad (2)$$

$$\mathrm{DG} + \mathrm{MeOH} \underset{k_4}{\overset{k_3}{\Leftrightarrow}} \mathrm{MG} + \mathrm{FAME} \quad (3)$$

$$\mathrm{MG} + \mathrm{MeOH} \underset{k_6}{\overset{k_5}{\Leftrightarrow}} \mathrm{GL} + \mathrm{FAME} \quad (4)$$

TG, MeOH, DG and FAME represent the concentration of triglycerides, methanol, diglycerides and fatty acid methyl ester, respectively. The rate constants for the forward and backward reactions of Equations (2)–(4) are depicted by k_1–k_6.

The reaction rate expressions for Equations (2)–(4) are presented in Equations (5)–(7).

$$r_1 = k_1 C_{TG}.C_{MeOH} - k_2\, C_{DG}\, C_{FAME} \quad (5)$$

$$r_2 = k_3 C_{DG}.C_{MeOH} - k_4 C_{MG} C_{FAME} \quad (6)$$

$$r_3 = k_5 C_{MG}.C_{MeOH} - k_6 C_{GL} C_{FAME} \quad (7)$$

The overall stoichiometry of the transesterification reaction is represented by Equation (8).

$$\mathrm{TG} + 3\mathrm{MeOH} \overset{\mathrm{H_2SO_4}}{=} 3\mathrm{FAME} + \mathrm{GL} \quad (8)$$

The reaction rate expression for Equation (8) is presented in Equation (9).

$$\frac{-dC_A}{dt} = k_f \,.\, C_A.C_B - k_b.C_C.C_D \tag{9}$$

C_A denotes the concentration of TGA in methanol/H_2SO_4 acid; C_B is the concentration of methyl alcohol; C_C and C_D are the concentrations of FAME and glycerol, respectively, formed during the reaction; k_f and k_b are forward and backward reaction rate constants respectively.

As the amount of methanol is in substantial excess, the concentration of methanol can be assumed to be constant with time.

Therefore, if "C_B" and "$C_{catalyst}$" are constant, we can write:

$$k'_f = k_f.C_B.C_{H2SO4} \tag{10}$$

where k'_f is the apparent forward rate constant and k_f is the fact forward rate constant.

Under these conditions, the reaction was assumed to be pseudo first order in the forward direction, due to the substantial excess of methanol, and second order in the reverse direction, hence conforming to the following kinetic law:

$$\frac{-dC_A}{dt} = k'_f \,.\, C_A - k_b.C_C.C_D \tag{11}$$

If C_C and C_D are assumed to be zero at the start (t = 0), and after time reaction are:

$$C_C = 3.(C_{AO} - C_A) \tag{12}$$

$$C_C = 3.(C_{AO} - C_{AO}\,(1 - X)) \tag{13}$$

$$C_C = 3.C_{AO}.X \tag{14}$$

$$C_D = C_{AO} - C_A \tag{15}$$

$$C_D = C_{AO}.X \tag{16}$$

Substitute Equations (14) and (16) into Equation (11):

$$\frac{-dC_A}{dt} = k'_f.C_A - k_b.(3.C_{AO}.X).(C_{AO}.X) \tag{17}$$

$$C_{AO}.\frac{dX}{dt} = k'_f \,.\, C_{AO}.\,(1 - X) - 3.k_b.C_{Ao}^2.X^2 \tag{18}$$

Rearrange Equation (18):

$$\frac{dX}{k'_f.(1 - X) - 3.k_b.C_{AO}.X^2} = dt \tag{19}$$

Take integration for Equation (19):

$$X(t) = \frac{-k'_f + \sqrt{k'_f}\,.\sqrt{12.C_{AO}.k_b + k'_f}\,.\tanh\left(\frac{1}{2}\left(\sqrt{k'_f}\,.\sqrt{12.C_{AO}.k_b + k'_f}\,.t + Arc\,.\tanh\left(\frac{\sqrt{k'_f}}{\sqrt{12.C_{AO}.k_b + k'_f}}\right)\right)\right)}{6.C_{AO}.k_b} \tag{20}$$

where, X is the percentage yield of FAME. The kinetic constants k'_f and k_b from Equation (20) were determined using the "DATA" solver in "EXCEL" as a nonlinear programming model and calculating the predicted yield% of FAME using the data as in sample of calculation for 700:1 methanol/oil mole ratio in Table 2. Comparison between predicted yield % and experimental yield% at a methanol/oil mole ratio of 700:1 and a temperature of 70 °C, is shown in Figure 5 with R^2 of 0.999.

Table 2. Calculation of data required for kinetic investigation.

Parameter (Unit)	Value
Chlorella vulgaris microalgae mass (g)	0.8
Oil content (%)	48
Mass of oil (g)	0.384
Molecular weight of oil (g/mole)	880
No. mole of oil (mole)	0.00044
Density of algae (g/mL)	0.896
Volume of algae (mL)	0.9
Catalyst type	H_2SO_4
Catalyst/oil molar ratio	8.5
No. mole of catalyst (mole)	0.0037
Molecular weight of catalyst (g/mole)	98.1
Mass of H_2SO_4 (g)	0.36
Density of catalyst (g/mL)	1.84
Volume of catalyst (mL)	0.2
Methannol/oil molar ratio	700
No. mole of methanol (mole)	0.308
Molecular weight of methanol (g/mole)	32.04
Mass of methanol (g)	9.86
Density of methanol (g/mL)	0.791
Volume of methanol (mL)	12.5
Total volume of reaction mixture (mL)	13.6
Initial oil concentration (mole/L)	0.032
Initial methanol concentration (mole/L)	24.6
Initial catalyst concentration (mole/L)	0.27

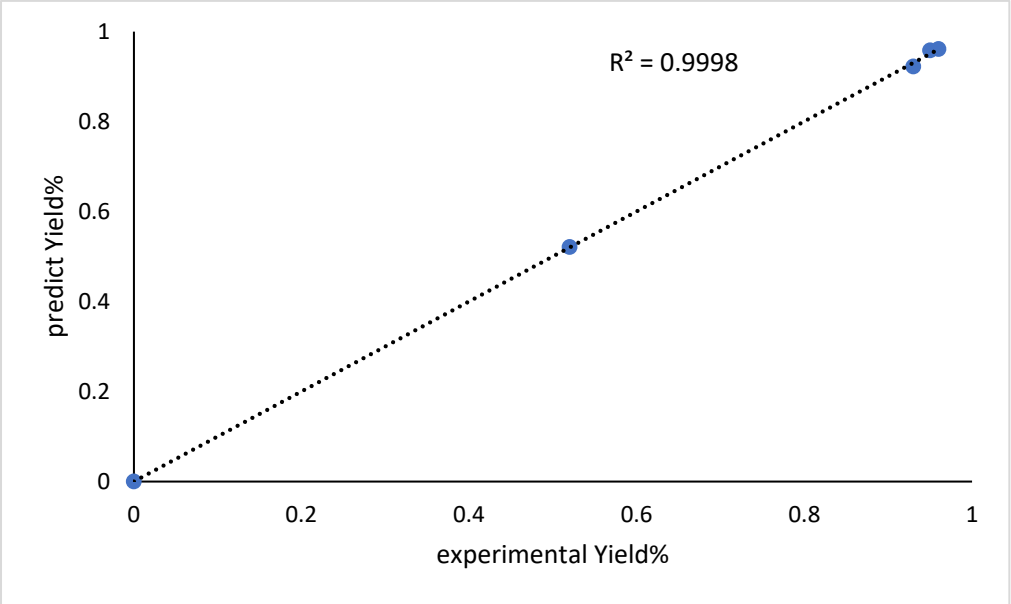


Figure 5. Comparison between the experimental data and kinetic model at a methanol/oil mole ratio of 700:1 and a temperature of 70 °C.

The modelled data was based on pseudo homogenous first order forward rate constants and second order backward rate constants, estimated from the data based on Equation (20), and is shown in Figure 6 with the experimental data obtained versus time at different methanol/oil mole ratios and temperatures. The figure shows the results and

demonstrates how well the reaction model fitted the data. The average of percentage root mean square error (%RMSE) of 0.5% is based on Equation (21).

$$\%\text{RMSE} = \left(\sqrt{\frac{\sum_{i=1}^{i=N}\left(x_{i\ exp.} - x_{i\ pred.}\right)^2}{N}}\right) \times 100 \quad (21)$$

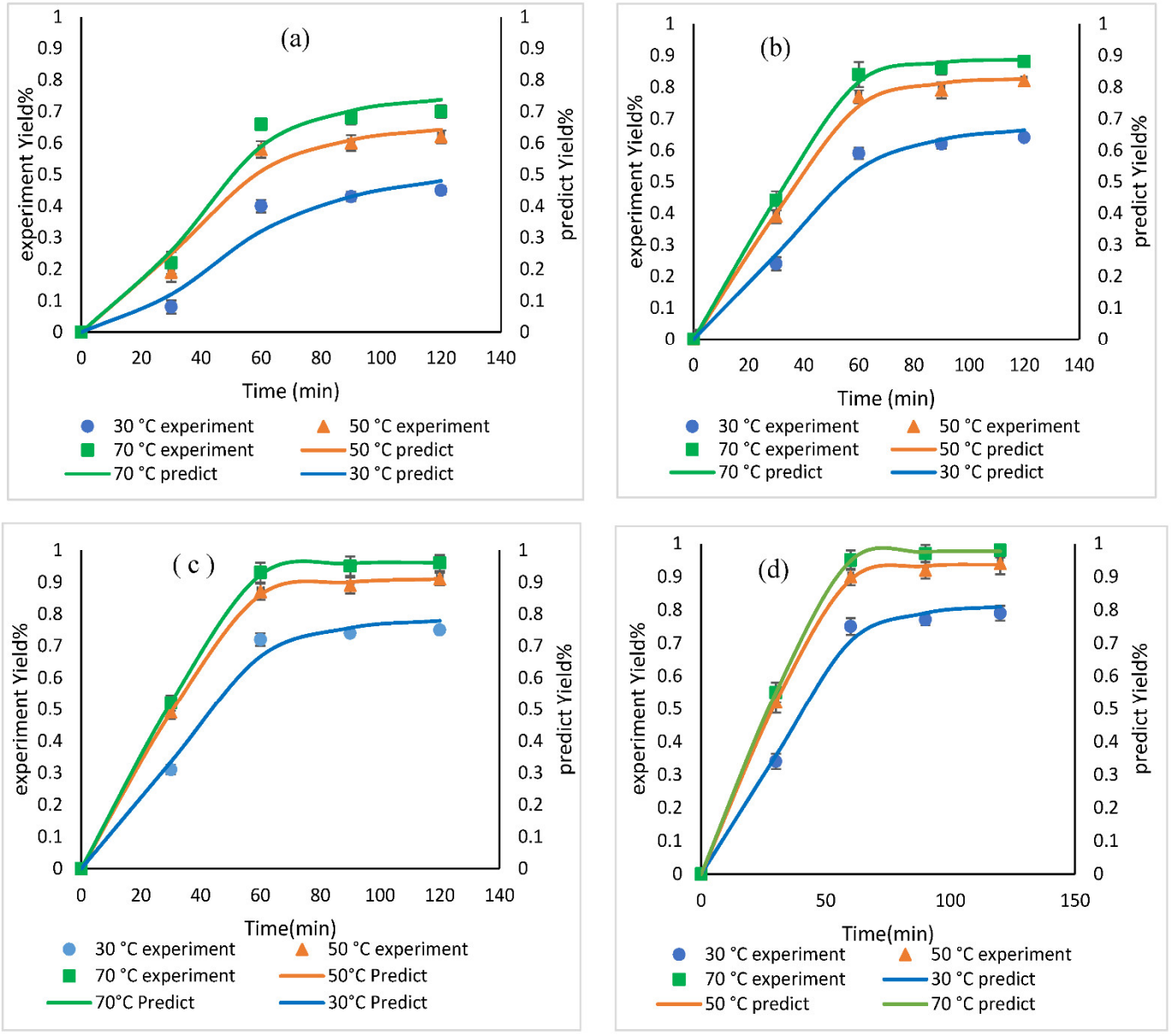


Figure 6. Experimental and mathematical yield% versus reaction times at different reaction temperatures for methanol/oil mole ratio: (**a**) 100:1, (**b**) 400:1, (**c**) 700:1 and (**d**) 1000:1.

The pseudo first order apparent and actual forward and second order backward rate constants obtained at 100:1 to 1000:1 methanol/oil mole ratio at 30 °C, 50 °C and 70 °C are shown in Table 3. The specific rate constant k_f was calculated from Equation (10). It can be seen that the forward rate constants increase with an increase in the reaction temperature, whereas backward rate constants decrease with an increase in the reaction temperature, which is consistent with that reported in the literature.

Table 3. Variation in rate constants with temperature and methanol/oil mole ratio.

MeOH/Oil Mole Ratio	Temperature (°C)	Apparent Pseudo First Order Forward Rate Constants, k_f' (min^{-1})	Actual Pseudo First Order Forward Rate Constants, k_f (min^{-1})	Pseudo Second Order Backward Rate Constants, k_b (L/mol.min)
100	30	0.0096	0.00049	0.0349
	50	0.0185	0.00095	0.0291
	70	0.0241	0.0012	0.0230
400	30	0.0203	0.0021	0.0887
	50	0.0383	0.0039	0.0585
	70	0.0493	0.0051	0.0428
700	30	0.0305	0.0049	0.1098
	50	0.0569	0.0093	0.0629
	70	0.0751	0.0123	0.0323
1000	30	0.0344	0.0073	0.1386
	50	0.0656	0.0140	0.0678
	70	0.0867	0.0186	0.0304

The kinetic model was developed with two parameters involving two steps, at the molar ratio MeOH/oil of 100:1 the mass transfer of triglycerides acid oil from algae to solvent methanol was controlled and at the molar ratios MeOH/oil of 400:1–1000:1, the reversible transesterification reaction of triglycerides acid oil to biodiesel was controlled. Therefore, k'_f at each temperature was calculated from average values k'_f for the chemical reaction step at 0.028 min^{-1}, 0.053 min^{-1} and 0.070 min^{-1} at 30 °C, 50 °C, and 70 °C respectively.

3.3. Determination of the Activation Energy

The rate constants obtained for the chemical reaction step as 0.005, 0.0089 and 0.012 at different reaction temperatures were employed to calculate the activation energy using the Arrhenius Equation (22).

$$k = Ae^{-Ea/RT} \tag{22}$$

where, Ea is the activation energy (J/mol), T is the absolute temperature (K), R is the universal gas constant (8.314 J/mol K), A is the pre-exponential factor (min^{-1}) and k is the reaction rate constant (min^{-1}).

Equation (22) can be expressed as Equation (23) by taking the natural logarithm:

$$lnk = lnA - \frac{Ea}{RT} \tag{23}$$

In Figure 7, a straight line is obtained from ln k'_f vs. 1/T plot. The slope depicts (-Ea/R) and the Arrhenius constant is represented by the y-intercept. Hence, the apparent activation energy (Ea) is calculated using the value obtained from the slope of the graph. The details of the pre-exponential factor and the activation energy that were obtained for the transesterification reaction of the *Chlorella vulgaris* microalgae to biodiesel by the acid catalysts are summarized in Table 4. The activation energy obtained in this study is much less than that reported in the literature. Baskar et al. [42] reported activation energy of 78.410 kJ/mol for transesterification of mahua oil to biodiesel using manganese doped zinc oxide nanocatalyst. Feyzi et al. [55] also reported activation energy of 72.86 kJ/mol for biodiesel production from sunflower oil using Al-Sr nanocatalysts. This is because these researchers used solid catalysts, rather than homogeneous, as here. The kinetic equation for this work is adequate in modeling the transesterification of the *Chlorella vulgaris* microalgae to biodiesel as indicated by R^2 of 0.998. The high R^2 implies that the data is well fitted to the kinetic model equation.

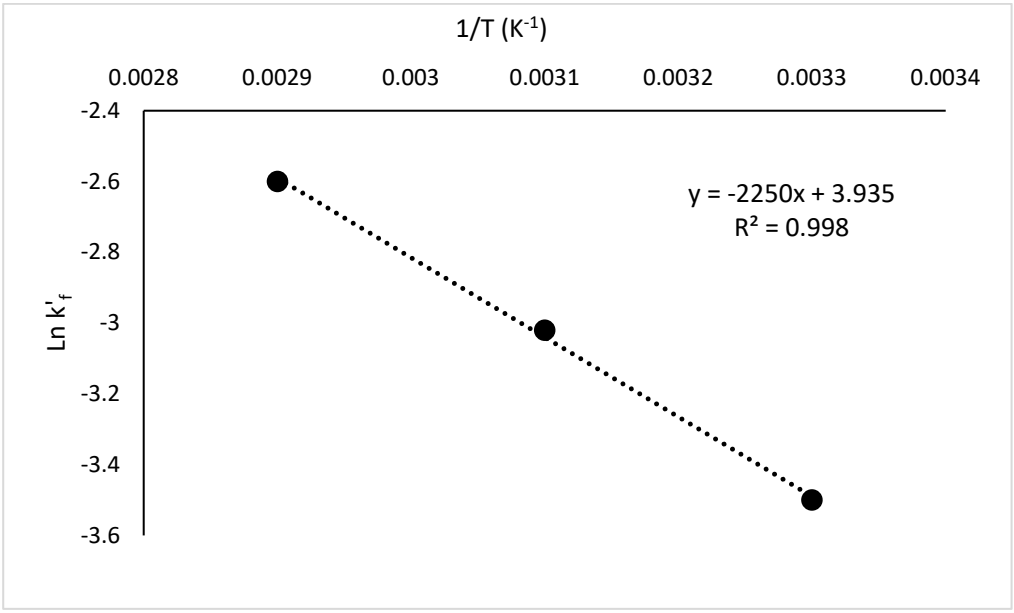


Figure 7. ln k'_f versus 1/T plot for Arrhenius constant and activation energy.

Table 4. Values of Arrhenius constant and activation energy.

Parameter	Value	Unit
Pre-exponential factor (*A*)	51.4	min^{-1}
activation energy (*Ea*)	18.7	kJ/mol

3.4. Thermodynamic Parameters for Microalgae Biodiesel Production

Thermodynamic parameters, such as enthalpy change (ΔH), Gibbs free energy (ΔG) and the entropy change (ΔS), are vital in determining the nature of the transesterification process involving the conversion of microalgae oil to biodiesel. The value of ΔG can be calculated using the Eyring–Polanyi equation, the transition state theory of thermodynamic interpretation can be written as given in Equation (24) to determine Gibbs free energy (ΔG):

$$k = \frac{k_B T}{h} \exp\left(-\frac{\Delta G}{RT}\right) \tag{24}$$

The change in Gibbs free energy can be calculated by Equation (25):

$$\Delta G = \Delta H - T.\Delta S \tag{25}$$

substituting Equation (25) into Equation (24):

$$k = \frac{k_B .T}{h} \exp\left(\frac{\Delta S}{R}\right). \exp\left(\frac{-\Delta H}{RT}\right) \tag{26}$$

Taking the natural logarithm of Equation (26):

$$\text{Ln}\left(\frac{K}{T}\right) = -\left(\frac{\Delta H}{RT}\right) + \left[\text{Ln}\left(\frac{k_B}{h}\right) + \left(\frac{\Delta S}{R}\right)\right] \tag{27}$$

where, k_B is the Boltzmann constant (1.38×10^{-23} J/K); h is Planck's constant (6.63×10^{-34} J s); R is the universal gas constant (8.314 J/mol.K); k is the apparent forward rate constant k'_f (min^{-1}); T is the absolute temperature (K); ΔH is the enthalpy change (J/mol); ΔS is entropy of activation (J/mol.K).

Using Equation (27) and the parameters of intercept and slope of the Eyring plot (Figure 8) between ln k'_f/T and 1/T, the values of ΔS, ΔH and ΔG were found as shown in Table 5.

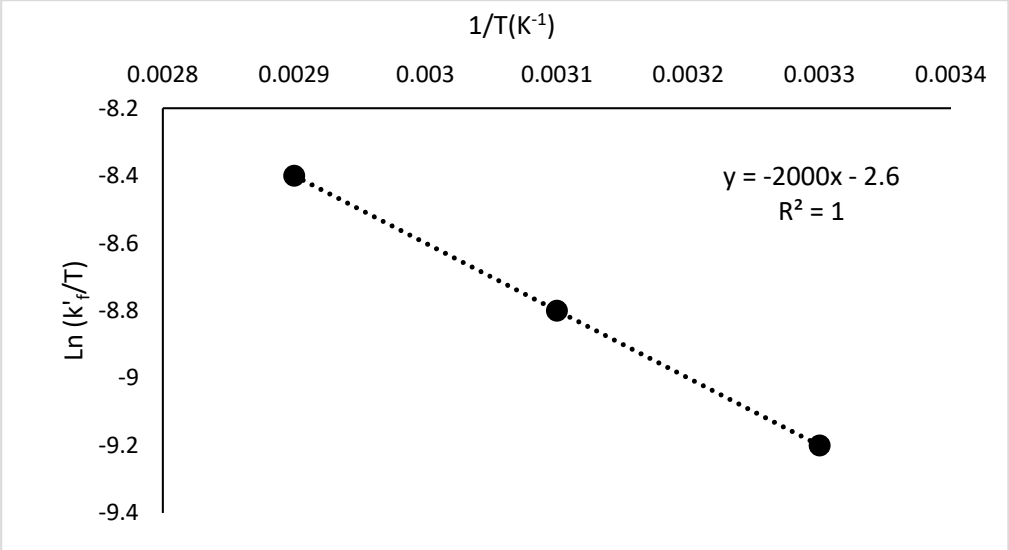


Figure 8. ln k'_f versus 1/T plot for Eyring thermodynamics parameters.

Table 5. Values of enthalpy and entropy change for *Chlorella vulgaris* in-situ transesterification process.

Thermodynamic Properties	Value	Unit
ΔH	16.63	kJ/mol
ΔS	−219.1	J/mol.K

From the obtained values of ΔS and ΔH, the values of ΔG were calculated at each temperature based on the relationship in Equation (25), as shown in Table 6.

Table 6. Values of Gibbs energy change at different reaction temperature of this work.

T (K)	ΔG (kJ/mol)
303.15	83.0
323.15	87.4
343.15	91.8

From Tables 5 and 6, it can be seen that the values of ΔH and ΔG are positive, while the ΔS is negative. A negative value of ΔS (<0) implies a better positioning of the reactants in the transition state compared to the reactants' conditions. A positive value of ΔG indicates that the state of the transesterification reaction is unspontaneous. The positive value of ΔH indicates that the transesterification reaction is endothermic, as established in the experimental runs. In a recent study [56], thermodynamic parameters such as ΔH, ΔS and ΔG were estimated as 55.09 kJ/mol, −0.103 kJ/mol.K and 89.90 kJ/mol, respectively for transesterification of waste cooking oil to biodiesel. The differences in the thermodynamic parameters could be attributed to the transesterification reaction conditions and the nature of the catalysts. The catalyst used in this study was NaOH. In a similar study [57], waste cooking and castor oil were used for the production of biodiesel, using potassium modified cerium oxide catalysts. Based on the thermodynamic analysis, the ΔH for the transesterification of the waste cooking and castor oil were estimated as 47.35 kJ/mol and 45.99 kJ/mol, respectively. The higher values are due to a heterogeneous catalyst being used. The ΔS for the transesterification of the waste cooking and castor oil were estimated as −128.69 J/K and −137 J/K, respectively. The ΔG for the transesterification of the waste cooking and castor oil were estimated as 90.85 kJ/mol and 92.66 kJ/mol, respectively. In a

separate study [43], *Spirulina platensis* algae biomass was used as the feedstock for biodiesel production by single stage extraction–transesterification with a homogenous acid catalyst. The values of thermodynamic parameters such as Gibbs free energy (ΔG), enthalpy of activation (ΔH) and entropy of activation (ΔS) were found to be 92.71 kJ/mol, 16.35 kJ/mol and −232.83 J/mol K, respectively.

4. Conclusions

This study has demonstrated that microalgal biodiesel can be made directly from the raw, untreated product of a foam column using an in situ reactive extraction technique catalyzed by sulfuric acid, despite the high water content of this foamate. The effects of reaction time (30–120) min, reaction temperature (30, 50, 70) °C and methanol/oil (100–1000) mole ratio on the biodiesel yield were investigated. The process was made more effective by cell wall lysis, which released a certain proportion of internal lipids. Beyond this, the biodiesel yield increased with increasing reaction time, temperature and methanol/oil mole ratio. The maximum biodiesel yield observed of 96% was obtained at a reaction time of 90 min, temperature of 70 °C and methanol/oil ratio of 1000. Methanol/oil ratios of 700+ and temperatures above 50 °C were required to achieve the desired yields of over 90%. The minimum molar ratio for the desired conversion should be used, as the most significant cost in a reactive extraction-based process is likely to be the distillation to separate and re-use the excess methanol. The shortest time possible should be used, to reduce the size and therefore the capital cost of the reactor. Therefore, the optimal conditions in this parameter space are 70 °C, 700:1 and 60 min.

Kinetic analysis of the reaction indicated that it could be modelled (R^2 = 0.99 as a first order pseudo forward and second order backward reaction, with an apparent activation energy of 18.7 kJ/mol. The transesterification reaction was found to be unspontaneous, endothermic and endergonic in nature.

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