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Optimizations of microwave-assisted extraction and transesterification of bio-crude oil from spirulina (*Arthrospira platensis*)

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Abstract−Optimizations of microwave-assisted extraction (MAE) and transesterification of bio-crude oil from spirulina (*Arthrospira platensis*) were conducted. The bio-crude oil from *A. platensis* was initially extracted using water as a solvent, centrifugation, dissolved with *n*-hexane, separation, and continued to evaporation. The optimization for the extraction was conducted with varieties of biomass-to-solvent ratio, extraction temperature, extraction time, and *n*-hexane contact time, yielding the optimum condition with biomass/solvent 1 : 7, extraction temperature 70 °C, extraction time 15 minutes, and contact time 25 minutes (D70/15-25) 5.56%. The average yield of the bio-crude oil obtained was 4.87%, variation 0.32, standard deviation 0.57, and standard error 0.14. The optimization for transesterification was carried out with variations of bio-crude oil-to-methanol ratio, reaction temperature, and reaction time, yielding the optimum condition with bio-crude oil/methanol 1 : 6, reaction temperature 65 °C, and reaction time 50 minutes (G65/50) 98%. The average yield of the biodiesel obtained was 88%, variation 50.29, standard deviation 7.09, and standard error 2.68. The cell damage before and after extraction and also conventional heating observed using SEM showed massive damage by this method.

Keywords: Bio-crude Oil, Biodiesel, MAE, Optimizations, Spirulina

INTRODUCTION

Global warming and fossil fuels exploitation have become ominous issues of the past couple of decades universally [1]. Carbon dioxide (CO_2) produced from combustions affects climate change [2]. Fossil fuel price has increased gradually and globally while sometimes experiencing fluctuation caused by political conflicts and a decrease in the amount of fuel supply. The current environmental conditions propel scientists to devise alternative energies that are renewable for the future [1]. One of which is the third generation biofuel from microalgae that helps to decrease CO_2 drastically [3-5]. In addition to its use of wastewater as a medium to live (land free), biomass is produced massively, faster than any other autotroph species, and it also helps preserve the ecosystem and environmental balance [6].

Arthrospira platensis or spirulina is a blue-green microalgae species, rich in thiamine, riboflavin, C-phycocyanin, A-phycocyanin, α-tocopherol, β-carotene, and essential fatty acids such as palmitic acid, linoleic acid, oleic acid, stearic acid, and γ-linoleic acid [7]. The range of lipids contained in the dry powder of *A. platensis* is around 5% [3], even though for many cases it yields 4-9% to 4-17% [8,9]. Finding the most effective method to extract bio-crude oil from *A. platensis* has become an interesting topic but yet has not been developed well thus far as most researches regarding *A. platensis* have focused only on C-phycocyanin pigment extraction [10,11], antioxidant activity [12], and polysaccharide [13].

The initial process to gain bio-crude oil from microalgae is by damaging the cells [14-16] to let the intracellular oil inside be extracted [17]. However, the existence of a thick cell wall of microalgae becomes an obstacle for the extraction process [1]. The most popular method of extraction, Soxhlet extraction, is considered to be less favorable as it requires very high energy, is time-consuming and harmful to the environment incited by the organic solvent used [18]. Thus, developments in technology, methodology, and innovation that prompt the use of less harmful chemicals should be conducted [19]. There have been many methods developed, such as acid addition, sonication, autoclaving, and bead-beating [20,21]. Nonetheless, microwave-assisted extraction (MAE) has proved to be the simplest, easiest, and most effective way to extract bio-crude oil from microalgae [15]. Studies of MAE for *A. platensis* using *n*-hexane, ether, chloroform, benzene, and ethanol as a single and combined solvents have previously been reported [22-24]. Therefore, the idea of extracting bio-crude oil from *A. platensis* using water as a solvent and MAE method should provide a solution and [18,25] becomes a support for green chemistry. Appertaining to it, the concept of bio-crude oil extraction from *A. platensis* using water as a solvent and MAE method should provide an opportunity in a green chemistry application [18,25].

MATERIALS AND METHODS

1. Microalgae and Material Sources

Spirulina powder was supplied from Nogotirto *Algae Park*, Universitas Gadjah Mada cultivated in Nogotirto Village, Gamping Subdistrict, Sleman Regency, Special Region of Yogyakarta. Methanol (CH_3OH), *n*-hexane (C_6H_{14}), and potassium hydroxide (KOH) were

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Table 1. Variations of MAE

Variations	I	II	III	IV	V
Biomass to solvent (g/mL)	1 : 10	1 : 9	1 : 8	1 : 7	1 : 6
Extraction temperatures (°C)	50	55	60	65	70
Extraction times (minutes)	5	10	15	20	25
Contact times (minutes)	5	10	15	20	25

Pro Analysis grade from Merck along with phenolphthalein indicator, sodium sulfate (Na_2SO_4) anhydrous, and universal indicator paper.

2. Extraction and Transesterification

(i) Microwave-assisted extraction for bio-crude oil: Initially, the dry powder of spirulina was extracted by MAE (*Electrolux*) using water (1 : 10 w/v) with extraction temperature of 50 °C for 5 minutes, *n*-hexane contact time of 5 minutes, and microwave power of 800 W (High). The extract was cooled and centrifuged with 3A and 2,000 rpm for 1 hour. The supernatant then was stirred with *n*-hexane for 5 minutes to dilute the existing bio-crude oil. The mixture was separated and the *n*-hexane was evaporated. The procedure was also done for other biomass-to-solvent ratios shown in Table 1. The percentage yield of bio-crude oil was calculated using Eq. (1).

$$\text{Yield of bio-crude oil (\%)} = \frac{\text{Grams of bio-crude oil obtained}}{\text{Grams of biomass used}} \times 100\% \quad (1)$$

(ii) Transesterification of bio-crude oil to biodiesel: The bio-crude oil (0.3 g) extracted from the optimum condition (D70/15-25) was refluxed with KOH dissolved in methanol (1 : 5 w/v) at 60 °C for 40 minutes, kept under room temperature, and then separated. The methyl ester (the upper layer) was washed using water and acetic acid (CH_3COOH) 1 M until neutral. The procedure was also done for bio-crude oil-to-methanol ratios of 1 : 6 and 1 : 7, reaction temperatures of 65 and 70 °C, and reaction times of 45 and 50 minutes. The percentage yield of biodiesel was calculated using the Eq. (2):

$$\text{Yield of biodiesel (\%)} = \frac{\text{Grams of biodiesel obtained}}{\text{Grams of bio-crude oil used}} \times 100\% \quad (2)$$

(iii) Average, variation, standard deviation, and standard error

Average (μ_x)

$$\mu_x = \Sigma_{i=1}^{n} \frac{x_i}{n} \quad (3)$$

Variation (σ^2)

$$\sigma^2 = \Sigma_{i=1}^{n} \frac{(x_i - \mu_x)^2}{n-1} \quad (4)$$

Standard deviation (σ)

$$\sigma = \sqrt{\Sigma_{i=1}^{n} \frac{(x_i - \mu_x)^2}{n-1}} \quad (5)$$

Standard error (δ)

$$\delta = \frac{\sigma}{\sqrt{n}} \quad (6)$$

3. Analyses and Characterization

3-1. Scanning Electron Microscopy (SEM) Analysis

The surface morphology before and after extraction and a comparison sample from conventional heating were characterized using SEM (*FEI, Inspect-S50*) (HV 20.00 kV, magnitude 5,000 times, WD 10.0 mm, spot 3.5 and 4.0, detector ETD, tilt 0°, and 20 μm) after being dried in an oven at 105 °C for 24 hours.

3-2. Free Fatty Acids (FFA) Percentage Determination

The FFA percentage of bio-crude oil was determined through titration (mixed with methanol and titrated with KOH 0.1 M) and calculated using Eq. (7):

$$\text{FFA (\%)} = \frac{M_{KOH} \times V_{KOH} \times MW_{palmitic\ acid}}{\text{Grams of bio-crude oil} \times 1{,}000} \times 100\% \quad (7)$$

3-3. β-Carotene Concentration Determination

The β-carotene concentration was determined using UV-Visible spectrophotometer (*Shimadzu UV-1700 Pharma*) (standard and sample were dissolved in ethanol, measured at λ 451 nm).

3-4. Fourier-transform Infrared (FTIR) Analysis

The vibration peaks were observed using FTIR (*Shimadzu IR Prestige-21*) in the wavenumber range of 4,000-400 cm^{-1} on NaCl plates for bio-crude oil and biodiesel, whereas for the dry spirulina powder KBr pellet was used.

3-5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS (*Shimadzu QP-2010S*) (column oven temp. 50 °C, injection temp. 300 °C, injection mode: split, flow control mode: pressure, pressure 13.0 kPa, total flow 79.3 mL/min, column flow 0.55 mL/min, linear velocity 26.8 cm/sec, purge flow 3.0 mL/min, and split ratio 139.0) analysis was conducted using BF_3 as an acid catalyst dissolved in methanol for the bio-crude oil.

3-6. ^1H and ^{13}C Nuclear Magnetic Resonance (NMR) Analysis

Samples were diluted in deuterated chloroform ($CDCl_3$) for the ^1H-NMR and ^{13}C-NMR (*JEOL Delta* and *Variant/Agilent*) (for ^1H NMR: field strength 11.747 T, acquired duration 1.746 s, domain 1H, freq. 500.160 MHz, offset 5.0 ppm, points 16,384, pre-scans 1, resolution 0.573 Hz, sweep 9.384 kHz, sweep clipped 7.508 kHz, and total scans 64); (for ^{13}C NMR: field strength 11.747 T, acquired duration 0.828 s, domain 13C, freq. 125.765 MHz, offset 100 ppm, points 32,768, pre-scans 4, resolution 1.207 Hz, sweep 39.557 kHz, sweep clipped 31.646 kHz, and total scans 1,024) analyses.

RESULTS AND DISCUSSION

1. Optimization of MAE of Bio-crude Oil

Polar molecules and ionic solutions absorb microwaves strongly as influenced by the existence of the permanent dipole moment. The effectiveness of the solvent used for MAE depends on the dissipation factor (tan δ), in which dielectric loss (ε_a) is supposed to be higher than the dielectric constant (ε_b). The higher the dissipation factor, the higher the ability of the solvent to dissipate the microwaves into heat [26]. If compared to methanol or any other solvents, the dissipation factor of water is rather low; however, environmental and economic aspects were weighed in this study. As opposed to conventional heating that uses a direct heating method, the microwave energy given in MAE induces polar molecules (in the solvent) to experience dipole rotation and ionic migration simulta-

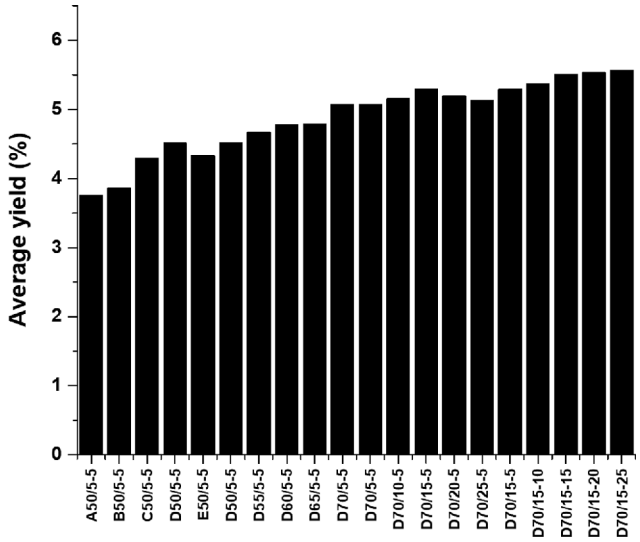


Fig. 1. The average yield for each variety of MAE parameter. *A (1 : 10); B (1 : 9); C (1 : 8); D (1 : 7); E (1 : 6) extraction temperature (°C)/extraction time (minutes)-contact time (minutes). A50/5-5 shows that extraction was conducted with 1 : 10 biomass/solvent ratio, 50 °C extraction temperature, 5 minutes extraction time, and 5 minutes *n*-hexane contact time.

neously (irradiation); thus, the gradient temperature is greatly reduced [26,27]. Contingent with a research studied by e Silva et al. [28], MAE method using water as a solvent and sample contact with ethanol could increase the yield obtained compared to when using methanol, ethyl acetate, and petroleum ether combined (1 : 1 : 1) 4.37% [29] and also nonpolar solvent 5.37% [30]. Water is used as a heating medium to extract the lipophilic functional compounds through destructing the bio-crude oil capsules in those very complex cell organs of the microalgae.

The single solvent used for MAE follows the type I mechanism [27]. Researches regarding *A. platensis* have been widely explored; however, the majority mainly focuses on C-phycocyanin, which is a very soluble blue pigment [10-12]. That being said, researches that focus on bio-crude oil are still rare. To improve the extraction process, the bio-crude oil extracted in a small amount was dissolved in *n*-hexane, a nonpolar substance compared to the ethanol used in another study [28]. Fig. 1 shows the average yield of MAE for the bio-crude oil of varying biomass-to-solvent ratio, extraction temperature, extraction time, and *n*-hexane contact time. The highest average yield of the bio-crude oil obtained was 5.56%; D70/15-25 with biomass-to-solvent ratio of 1 : 7, extraction temperature of 70 °C, extraction time of 15 minutes, and *n*-hexane contact time of 25 minutes.

Average, Variation, Standard Deviation, and Standard Error

The average yield of the bio-crude oil obtained was 4.87%, variation 0.32, standard deviation 0.57, and standard error 0.14.

2. SEM Surface Morphology Analysis and Comparison Before and After Extraction

Surface morphology comparison between samples extricated through heat reflux extraction (HRE) and MAE was observed using SEM, wherein the cell damage was highly prominent in the sample that underwent MAE compared to the sample before treatment and the sample subjected to HRE using the plant cells [27]. The morphology comparison of the sample before and after treatment is shown in Fig. 2. The change in morphology after MAE (c) appears to be more evident than after HRE (b), a method based on the previous study [27], whereby the topology of cells before treatment (a) was very harsh. The degree of cell damage is a factor in the effectiveness of MAE which is shown from the white circle mark in each figure in Fig. 2.

3. Determination of Free Fatty Acids (FFA) Content of Bio-crude Oil

Base or acid catalysts can catalyze transesterification or esterification [31], or even a double transesterification (esterification followed by transesterification) when the FFA content is 2% or above. The initial esterification is done to reduce FFA content (slower process and consumed more methanol using acid catalyst) to then be continued onto transesterification (faster process and irreversible reaction using base catalyst) in order to prevent saponification and simplify the separation process [32]. The average FFA content of bio-crude oil determined through titration was 1.41% indicating that transesterification could be well conducted using base catalyst without a double transesterification.

Haruna et al. [33] concluded that the higher the FFA content, the lower the biodiesel yield produced for *Jatropha curcas*, when using a base as a catalyst, in which each FFA content of 0.22; 1.00; and 3.92% yielded 99.99; 99.11; and 94.76% of biodiesel, respectively.

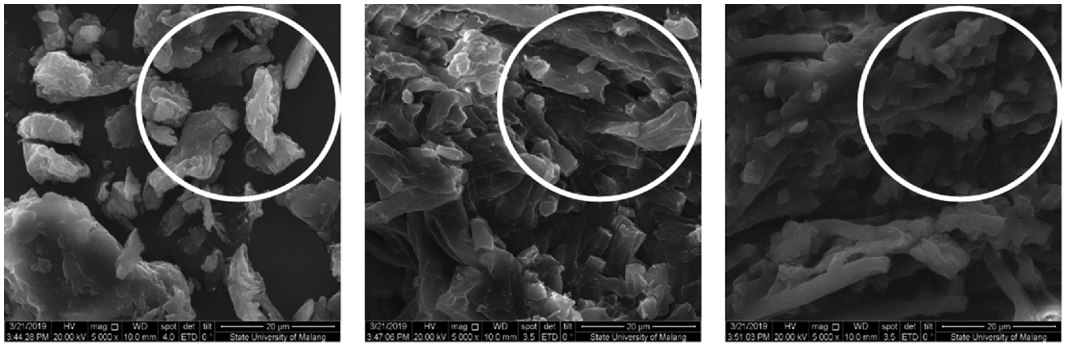


Fig. 2. The morphology of (a) before treatment, (b) after conventional heating, and (c) after MAE samples.

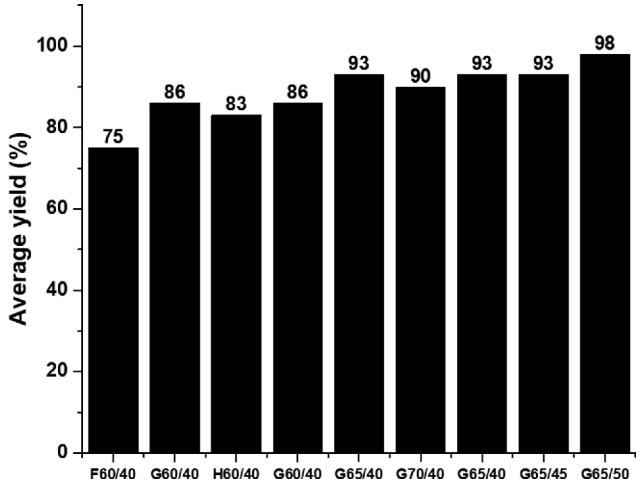


Fig. 3. The average yield for each variety of transesterification parameter. *F (1 : 5); G (1 : 6); H (1 : 7) reaction temperature (°C)/reaction time (minutes). F60/40 shows that reaction was conducted with 1 : 5 bio-crude oil/methanol ratio, 60 °C reaction temperature, and 40 minutes reaction time.

4. Determination of β-Carotene Concentration in Bio-crude Oil

The β-carotene belongs to the most popular form of carotenoid, and since it is a lipophilic compound it dissolves in oil. Its concentration can be determined by measuring the bio-crude oil with absorption at λ 451 nm and calculating it according to the standard solution equation. The concentration of β-carotene found was 6.63 μg/mL, which is lower in comparison to the result in a previous study [29]. This has to do with the fact that *A. platensis* may be different from one to another according to the growing medium, condition, and nutrition.

5. Optimization of Transesterification of Bio-crude Oil

Fig. 3 shows the average yield of biodiesel from the transesterification of bio-crude oil with variations of bio-crude oil-to-methanol ratio, reaction temperature, and reaction time. The highest average yield of biodiesel obtained was 98%; G65/50 with 1 : 6 bio-crude oil-to-methanol ratio, 65 °C reaction temperature, and 50 minutes reaction time.

Average, Variation, Standard Deviation, and Standard Error

The average yield of the biodiesel obtained was 88%, variation 50.29, standard deviation 7.09, and standard error 2.68.

The viscosity of bio-crude oil is 10 times higher than regular fuels, thus causing imperfect combustion and carbon deposition in the injector leading to engine fouling [34]. Apart from mixing with diesel, emulsification, pyrolysis, and cracking, transesterification seems to be more profitable because it produces glycerol as a side product [32,34]. Transesterification should be water-free [35], not neglecting that KOH needs to be dissolved directly into methanol using analysis grade chemicals.

6. Characterization

6-1. FTIR Analysis

6-1-1. FTIR Analysis of *A. platensis* Dry Powder

The peaks in Fig. 4(a) at 2,927 to 2,860 cm^{-1} are the vibrations of asymmetric and symmetric stretching of CH_2 ($\nu_{as}CH_2$ and ν_sCH_2),

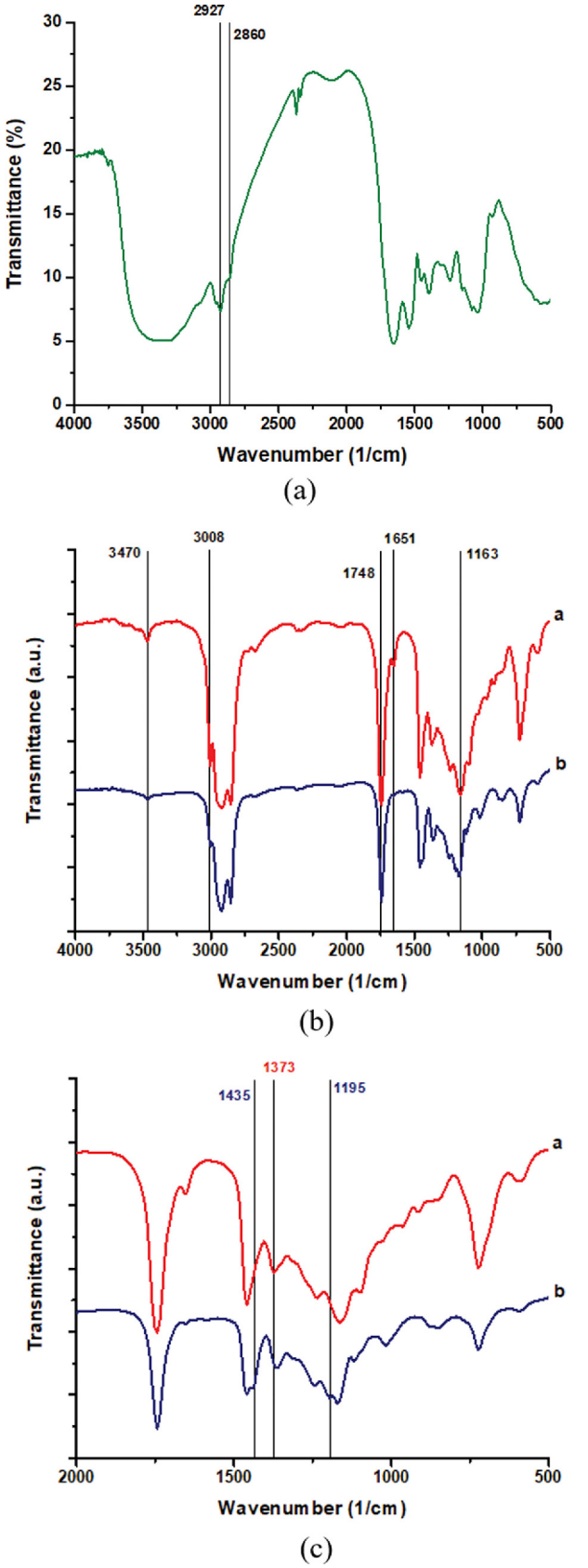


Fig. 4. FTIR analysis of (a) *A. platensis* powder, (b) overall vibrations, and (c) typical vibrations of bio-crude oil and biodiesel. *Script a (red) is bio-crude oil and b (blue) is biodiesel.

Table 2. FTIR vibrations of bio-crude oil and biodiesel

Bio-crude oil		Biodiesel	
Wavenumber (cm^{-1})	Vibration	Wavenumber (cm^{-1})	Vibration
3,471	νO-H	3,464	νO-H
3,008	νC_{sp^2}-H	3,008	νC_{sp^2}-H
2,924	$\nu_{as}CH_2$	2,924	$\nu_{as}CH_2$
2,854	$\nu_s CH_2$	2,854	$\nu_s CH_2$
1,743	ν_{as}C=O	1,743	ν_{as}C=O
1,651	νC=C	1,658	νC=C
1,458	$\delta_s CH_2$	1,458	$\delta_s CH_2$
-	-	1,435	$\nu_{as}CH_3$
1,373	νO-CH_2	-	-
-	-	1,195	νO-CH_3
1,165	ν_{as}C-O	1,172	ν_{as}C-O
725	ρCH_2	725	ρCH_2

indicating the existence of lipid [36]. The FTIR analysis aimed to confirm that bio-crude oil was able to be extracted.

6-1-2. FTIR Analysis of Bio-crude Oil

The peaks in Fig. 4(b); script a, identified for bio-crude oil with a broad peak at ν 3,471 cm^{-1} (νO-H), three adjacent peaks (intense peak at 3,008 cm^{-1} (νC_{sp^2}-H), 2,924 cm^{-1} ($\nu_{as}CH_2$), and 2,854 cm^{-1} ($\nu_s CH_2$)), a sharp peak at 1,743 cm^{-1} (ν_{as}C=O), 1,651 cm^{-1} (νC=C), 1,165 cm^{-1} (ν_{as}C-O), and a very typical peak at 1,373 cm^{-1} (νO-CH_2) of a triglyceride of bio-crude oil. The peaks at 1,458 cm^{-1} ($\delta_s CH_2$) and 725 cm^{-1} are *in-plane bending vibration* specified as *rocking* (ρCH_2). The identification of functional groups refers to [37,38] and is shown in more detail in Table 2.

6-1-3. FTIR Analysis of Biodiesel

The peaks in Fig. 4(b); script b, identified for biodiesel with a broad peak at ν 3,464 cm^{-1} (νO-H), three adjacent peaks (intense peak at 3,008 cm^{-1} (νC_{sp^2}-H), 2,924 cm^{-1} ($\nu_{as}CH_2$), and 2,854 cm^{-1} ($\nu_s CH_2$)), a sharp peak at 1,743 cm^{-1} (ν_{as}C=O), 1,658 cm^{-1} (νC=C), 1,172 cm^{-1} (ν_{as}C-O), and very typical peaks at 1,195 (νO-CH_3) and 1,435 cm^{-1} ($\nu_{as}CH_3$) of a methyl ester of biodiesel. The peaks at 1,458 cm^{-1} ($\delta_s CH_2$) and 725 cm^{-1} are *in-plane bending vibration* specified as *rocking* (ρCH_2). The identification of functional groups refers to [37,38] and is shown in more detail in Table 2.

The reduction of the transmittance intensity of biodiesel (script b) from bio-crude oil (script a) in Fig. 4(c) is due to transesterification, where some methyl functional groups were added resulting in the appearance of different and strange vibrations appeared that decreased the intensity.

6-2. GC-MS Analysis

6-2-1. GC-MS Analysis of Bio-crude Oil

The fatty acid methyl esters (FAMEs) were determined by converting the bio-crude oil through esterification or transesterification, where each kind of FAME is equivalent or correlates to its fatty acid. This conversion is intended to decrease the boiling point of bio-crude oil so it will become volatile enough to vaporize in GC column and be detected by MS.

A. platensis contains some essential fatty acids, such as palmitic acid, linoleic acid, stearic acid, and γ-linoleic acid [7]. The fatty acids detected by MS detector are oleic acid (34.27%), palmitic acid (34.06%), linoleic acid (25.59%), and stearic acid (6.09%). The GC spectra are shown in Fig. 5(a) and outlined in more detail in Table 3. The result depicts varying fatty acids composition in conformity with [39] *in situ* transesterification study reporting that the fatty acids obtained were capric acid (38.70%), linoleic acid (13.70%), α-linoleic acid (9.56%), oleic acid (14.00%), palmitic acid (11.50%), butyric acid (6.52%), caprylic acid (3.40%), and stearic acid (2.66%), along with a study from Esquivel-Hernández et al. [29] claiming the presence of palmitic acid (52.64%), linoleic acid (28.15%), γ-

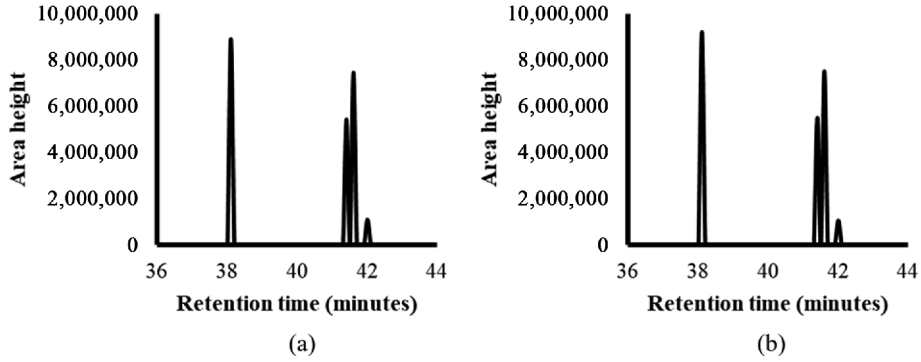


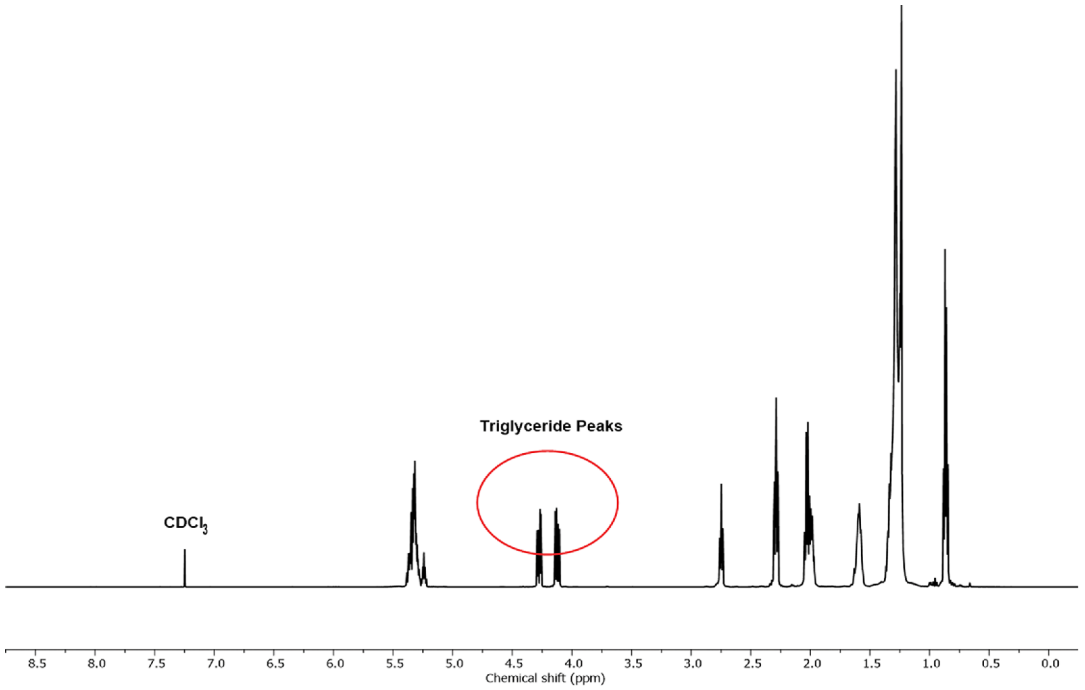
Fig. 5. GC peaks of (a) methyl ester of bio-crude oil and (b) biodiesel.

Table 3. FAMEs content and retention time of bio-crude oil

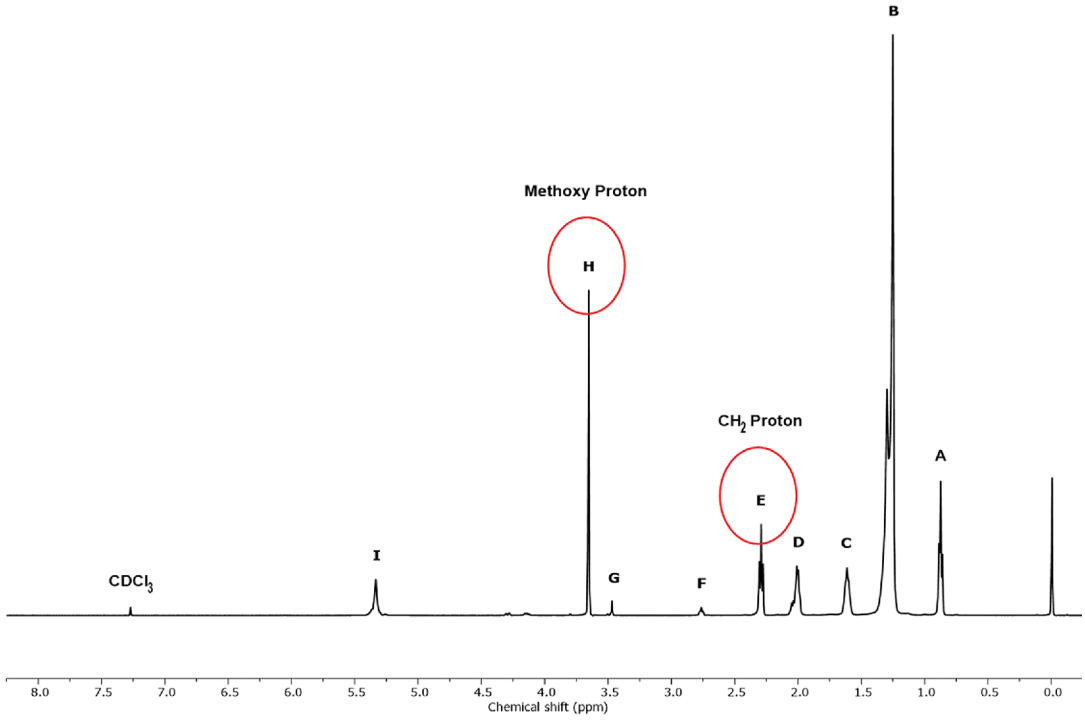
Peak	Retention time (minutes)	Compound name	Compound formula	Content (%)
1	38.104	Methyl palmitate	$C_{17}H_{34}O_2$	34.06
2	41.496	Methyl linoleate	$C_{19}H_{34}O_2$	25.59
3	41.628	Methyl oleate	$C_{19}H_{36}O_2$	34.27
4	42.098	Methyl stearate	$C_{19}H_{38}O_2$	6.09

Table 4. FAMEs content and retention time of biodiesel

Peak	Retention time (minutes)	Compound name	Compound formula	Content (%)
1	38.104	Methyl palmitate	$C_{17}H_{34}O_2$	35.00
2	41.496	Methyl linoleate	$C_{19}H_{34}O_2$	25.41
3	41.628	Methyl oleate	$C_{19}H_{36}O_2$	34.33
4	42.098	Methyl stearate	$C_{19}H_{38}O_2$	5.26

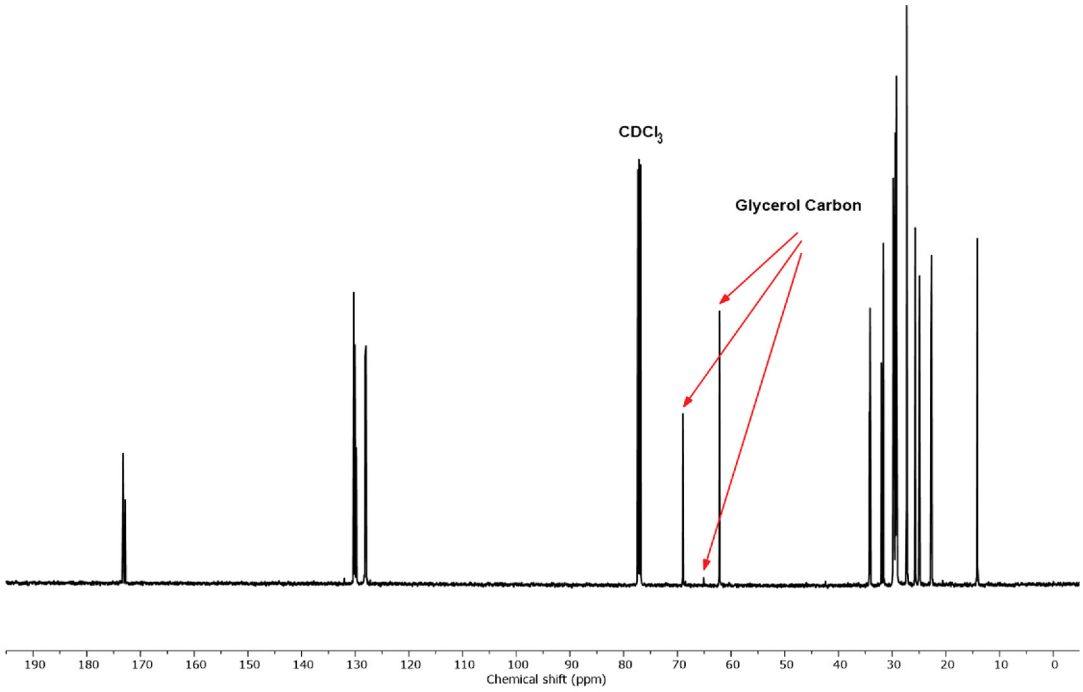


(a)

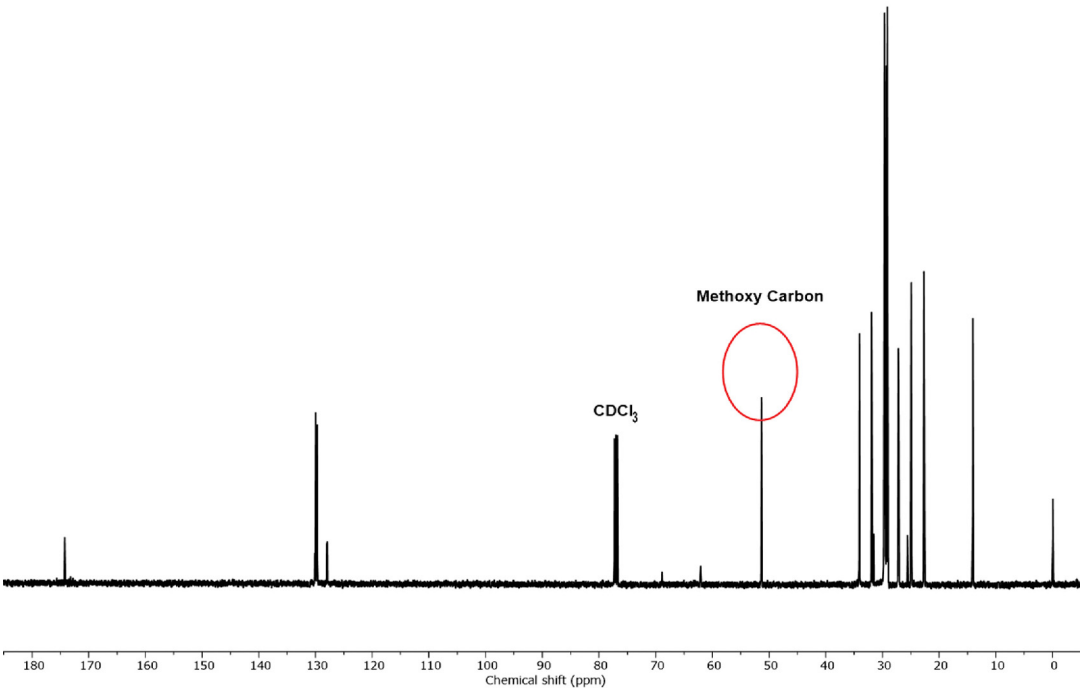


(b)

Fig. 6. ^{1}H-NMR spectra of (a) bio-crude oil and (b) biodiesel as well as ^{13}C-NMR spectra of (c) bio-crude oil and (d) biodiesel.



(c)



(d)

Fig. 6. Continued.

linoleic acid (15.47%), and palmitoleic acid (3.72%).

6-2-2. GC-MS Analysis of Biodiesel

The analysis of GC-MS for biodiesel exhibits the same FAMEs as contained in the bio-crude oil. The fatty acids are oleic acid (34.33%), palmitic acid (35.00%), linoleic acid (25.41%), and stearic acid (5.26%). The GC spectra are shown in Fig. 5(b) and outlined in more detail in Table 4. The difference in percentage may be due to the catalyst used, wherein the conversion of bio-crude oil to FAMEs, acid catalyst (BF_3) was used, while for biodiesel, transesterification using base catalyst (KOH) was applied.

6-3. ^{1}H-NMR and ^{13}C-NMR Analysis

6-3-1. ^{1}H-NMR Analysis of Bio-crude Oil

The multiplet peaks at δ 4.31 and 5.33 ppm on ^{1}H-NMR spectra indicate the existence of glyceride proton and olefinic hydrogen [37], confirming triglyceride presence in the bio-crude oil. Fig. 6(a) shows ^{1}H-NMR spectra for the bio-crude oil.

Table 5. Chemical shifts observed in ^{1}H-NMR spectra of biodiesel

Chemical shift (δ) (ppm)	Multiplicity	Functional group	Code
0.80	Triplet, 3H	-CH_3	A
1.20	Multiplet, 2H	-$(CH_2)_4$-	B
1.60	Multiplet, 2H	β-CH_2-CH_2-	C
2.00	Multiplet, 2H	-CH_2-CH=CH-	D
2.31	Triplet, 2H	α-CH_2-COO-	E
2.80	Multiplet, 2H	-CH_2-CH=C=CH-	F
3.59	Multiplet, 1H	-CH-	G
3.66	Singlet, 3H	-OCH_3	H
5.30	Multiplet, 1H	-CH=CH-	I

Table 6. Chemical shifts observed in ^{13}C-NMR spectra of biodiesel

Chemical shift (δ) (ppm)	Functional group
13.5	-CH_3
22-33.5	α-CH_2-COO-
51.41	-OCH_3
127.8-129.7	CH_3-COO-CH=CH-
131.88-127.08	CH_3-COO-CH=C=CH-
174	-CH_2-COO-CH_2-

6-3-2. ^{1}H-NMR Analysis of Biodiesel

The ^{1}H-NMR spectra show the success of the transesterification as a single and sharp peak at δ 3.66 ppm, identified as methoxy proton followed by the disappearance of peaks at 2.31 (triplet); 4.31 ppm; and 5.33 ppm (triplet) that indicate α-CH_2 proton [37]. Table 5 and Fig. 6(b) show the chemical shifts observed and ^{1}H-NMR spectra for the biodiesel.

6-3-3. ^{13}C-NMR Analysis of Bio-crude Oil

Peaks appear at δ 60.26, 61.94, and 68.69 ppm, indicating the existence of glycerol carbon atoms [40]. Fig. 6(c) shows ^{13}C-NMR spectra for the bio-crude oil.

6-3-4. ^{13}C-NMR Analysis of Biodiesel

The disappearance of peaks of glycerol at δ 60.26, 61.94, 68.69 ppm followed by the appearance of a peak at 51.41 ppm indicates the existence of methoxy carbon in methyl ester [40]. Table 6 and Fig. 6(d) show the chemical shifts observed and ^{13}C-NMR spectra for the biodiesel.

CONCLUSIONS

The optimum MAE result was achieved with D70/15-25; 5.56%, the average yield of the bio-crude oil 4.87%, variation 0.32, standard deviation 0.57, and standard error 0.14, using water as a single solvent following the type I mechanism. For transesterification it was G65/50; 98%; the average yield of the biodiesel obtained was 88%, variation 50.29, standard deviation 7.09, and standard error 2.68. The cell damage after MAE can be identified compared to before MAE and after conventional heating. The amount of FFA content with a value lower than 2% determined the allowance use of a base catalyst in the transesterification. All the characterizations concluded the presence of triglyceride in the bio-crude oil and methyl ester in the biodiesel. The spirulina oil in a certain area and different nutrition provided during the growth determined its specific and unique compounds. In an economical-based industry, *A. platensis* was considered to be lacked oil produced; however, a more developed study is needed to find a better extraction method since the percentage of bio-crude oil in this microalgae varies very differently in many preceding studies.

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