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Biodiesel production from marine microalgae *Nannochloropsis gaditana* by *in situ* transesterification process

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Microalgae is one of the best sources of renewable energy production, such as biofuels. The production of biodiesel from microalgae has several advantages, including the high productivity of lipid and the possibility of cultivating them on marginal land. One of the challanges in using microalgae for biodiesel production is the complexities process of lipids extraction by organic solvents followed by transesterification. The aim of this work is to optimize this process by a single extraction and conversion step. The reaction was carried out for different parameters such as; various oil to methanol ratios, concentration of catalyst, temperature and time reaction. The lipid content of *Nannochloropsis gaditana* microalgae was 0.19 g/g biomass. The best yield of fatty acid methyl ester (65.6%) was obtained at 150 min duration for algae drying, 60% (wt./wt. oil) H_2SO_4 as catalyst concentration, and 1:8 algae biomass to methanol ratio (w/v). The algal biodiesel samples were analyzed with gas chromatography mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FT-IR). *N. gaditana* microalgae investigated in this study, proved to be suitable as raw material for biodiesel production, due to their high cetane number (69.68). From the FT-IR result and fatty acid profile, it was implied that marine microalgae, *N. gaditana* in this study can be considered as potential feedstock for biodiesel production to fight the future energy crisis.

Key words: Biodiesel, fatty acid methyl ester, microalgae, *Nannochloropsis gaditana*, transesterification.

INTRODUCTION

The vegetable-oil derivative “biodiesel” offers several advantages as an alternative fuel for diesel engines (Maher et al., 2016). These include improved fuel performance and lubricity, a higher cetane rating than

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petrol-diesel, a higher flashpoint that makes it safe to handle, lower toxicity to plants and animals, reduced exhaust emissions, and the fact that it is simple to phase in and out of use (Sivaprakasam and Saravanan, 2007). It is a local renewable source of energy and highly biodegradable (Meng et al., 2008).

Microalgae are regarded as a promising source of biofuels due to their high lipid contents, high growth rates and requirement of smaller cultivation area. However, the production of bioenergy by the microalgae is still uneconomical. In fact, one of the production steps of transesterification which consumes the most energy is the step of lipid extraction from microalgae cells (Chattip et al., 2012; Meo et al., 2017).

In recent years, many studies tried extensively to improve the transesterification process by the variation of the reaction conditions, for exemple, the choice of catalyst, oil/alcohol ratio, temperature and reaction time (Bradley et al., 2011; Vlada et al., 2014; Verma and Barrow, 2015; Veillette et al., 2017). Although, this research aims to improve the lipid extraction method, others propose a rather interesting alternative approach to *in situ* transesterification without going through a tedious extraction step. Therefore, it would be more interesting to extract and convert the triglycerides from the microalgae into biodiesel in a single step, avoiding the use of large amounts of organic solvents (Chattip et al., 2012).

The *in situ* transesterification methods have been evaluated for biodiesel production from various raw materials, such as vegetable oil (Haas et al., 2004), wastewater sludges (Mondala et al., 2009), rice bran oil (Shiu et al., 2010), cotton seed oil (Qian et al., 2008) and microalgae (Li et al., 2011). It was reported that the direct method may result in higher yields of fatty acid methyl ester (FAME) than those obtained with the two-stage extraction (Bradley et al., 2011).

The *in situ* transesterification process, like conventional reaction, uses acid, basic or anzymatic catalysts. An acid catalyst (sulfuric acid) was chosen for this study because the acid catalyzed reactions were found to be affective in converting the free fatty acids and the triglycerides into FAME. In fact, the use of acid catalyst is the most appropriate method for organic substrates. It give very high yields in FAME (up to 99% of conversion) (Bharathiraja et al., 2014).

The aim of this work is to study the process of direct transesterification by optimizing the extraction and conversions step of marine microalgae, *Nannochloropsis gaditana*. It is important to study the production of microalgae biodiesel in Algeria, where pollution is becoming more and more widespread, due to the waste generation by the petroleum industries. It is also interesting to use the microalgae as an alternative source of biofuel. For the transesterification optimization, variations of the reaction conditions were carried out such as: 1) catalyst concentration; 2) reacting alcohol volume; 3) temperature and 4) reaction time. The study objectives also include the characterization of biodiesel produced by gas chromatography mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FT-IR).

MATERIALS AND METHODS

Biological materials

The dried and powdered biomass of *N. gaditana* was used as a biological material in this study. It was provided by the company, Partisano Biotech Algeria in Oran.

Estimation of total lipid

The lipid content of *N. gaditana* was estimated by Soxhlet method of Schafer (1998). 6 g of dried sample was placed in a porous cellulose thimble (25 × 80 mm). The extraction was carried out in Soxhlet apparatus for 4 h. The system was equipped by water-cooled condenser suspended above a 500 ml flask containing 250 ml hexane: 2-propanol (2:1). The solvent was evaporated (55°C) and the lipids content was calculated as a percentage of the dry weight of the algae.

In situ transesterification of microalgal lipid

The transesterification process was conducted simultaneously with oil extraction. This method was based on Nautiyal et al. (2014) protocol with some modifications. The dried algae was subjected to pulverization in mortar for cell distruction. 5 g of resultant powdered was placed in the 100 ml flask with hexane and methanol at various algal biomass to methanol w/v ratios (1:6, 1:8 and 1:10) and different concentration of sulfuric acid H_2SO_4 (40, 60 and 80% by weight of algae). The magnetic stirrer was used to stir the contents in the reactor, the mixture was hearted to 90°C, and the reaction was tested for 30, 90 or 150 min.

The system was equipped with a condenser to maintain the atmospheric pressure inside the reaction and to avoid loss of solvents by evaporation. At the end of the reaction in each experiment, the products were centrifuged at 5000 rpm for 10 min and pouder into a separating funnel. The biodiesel layer (Top layer) was washed with distilled water (30% v/v) and the solvent was evaporated. Biodiesel was heated at 100°C for 15 min to remove water and excess solvents. The gravimetric method was used to determine the biodiesel content and the yield was calculated using Equation 1:

Biodiesel yield (%) = [Biodiesel produced (grams) / Oil produced (grams)] x 100 1

Analysis of biodiesel

Gas chromatography mass spectrometry (GC-MS) analysis

FAMEs were analyzed by GC-MS carried out on a PerkinElmer Clarus 500 Gas Chromatograph couplet to Clarus 500 Mass Spectrometer with liquid autosampler (capillary column: 30 m, 0.25 μm diameter). Sample injected (2 μl) took place at 50°C temperature and was held for 3 min. Then, the temperature increased up to 280°C at 10°C min^{-1} and for 3 min. The vector gas used was helium. The average molecular weight of the oil (MM_{oil})

Table 1. Molecular weight and mass contribution of fatty acids of the obtained biodiesel from the promising green microalga, *N. gaditana* using GC-MS.

Fatty acid methyl ester (FAME)	Molecular mass (g/mol)[a] (MM)	Distribution in sample (%)[b]	Molecular mass contribution (g/mol) (MMc) (aXb)/100
(C8:0) Methyl octanoate	158.2	0.23	0.36
(C9:0) Methyl nonanoate	172.2	0.02	0.03
(C10:0) Methyl decanoate	186.2	0.19	0.35
(C11:0) Methyl undecanoate	200.3	ND	ND
(C12:0) Methyl laurate (X_1)	214.3	0.01	0.02
(C13:0) Methyl tridecanoate	228.3	0.97	2,21
(C14:0) Methyl myristate (X_2)	242.4	7	16,96
(C16:0) Methyl palmitate (X_3)	270.4	76.98	208.15
(C16:1) Methyl palmitoleate (X_4)	268.4	ND	ND
(C18:0) Methyl stearate (X_5)	298.5	2.60	7.76
(C18:1) Methyl oleate (X_6)	296.4	0.90	2.66
(C18:2) Methyl linoleate (X_7)	294.4	9.44	27.79
(C18:3) Methyl linolenate (X_8)	292.4	1.62	4,73
Total saturated fatty acids		88.04	
Total unsaturated fatty acids		11.96	
Average molecular mass of constituent fatty acids (MM_{FA})			271.02

was determined using Equation 2:

$$MM_{oil}=[3MM_{FA} + MM_{Glycerol}] - 3MM_{OH,H} \quad (2)$$

Where, $MM_{Glycerol}$ represents the molecular weight of glycerol and $MM_{OH,H}$ represents the molecular weight of OH group and hydrogen (El-Shimi et al*.,* 2013).

The molecular weight of biofuel was determined using Equation 3 (EL-Shimi et al., 2013):

$$MM_{FAME} = MM_{FA} + 15 \quad (3)$$

The cetane number (CN) of biofuel was calculated using Equation 4 (Bamgboye and Hansen, 2008):

$$CN = 61.1 + 0.0088X_2 + 0.133X_3 + 0.152X_4 - 0.101X_5 - 0.039X_6 - 0.243X_7 - 0.395X_8 \quad (4)$$

Where, CN: cetane number, X_1, X_2.....X_8, are % compositions of FAME shown in Table 1.

Fourier transform infrared spectroscopy (FT-IR) analysis

The biodiesel samples were measured on FT-IR alpha Bruker. The resolutions of 26 scans were taken and the sample was recorded in the range of 4000 to 500 cm^{-1}.

Statistical analysis

The experiments were carried out in triplicate. The average of the three values obtened was used to calculate the standard deviation (SD). The final values were represented by mean ± SD. The statistical analysis consists of a parametric test of ANOVA 1 (Tukey HSD) or a non-parametric test of Kruskal-Wallis (Mann-Witney). P values ≤ 0.05 were regarded as statistically significant.

RESULTS AND DISCUSSION

Total lipid content

The total lipid content of *N. gaditana* biomass was found to be 19.18 ± 0.4% which was determined by the method of Shafer (1998). Abubakar et al. (2012) reported that the lipid content in *Chlorella* species presents hight oil yields (10.5%) followed by *Euglena acus* (5.78%), *Nitzschia* (3.63%), *Ankistrodesmus falcatus* (1.58%) and *Scenedesmus acuminatus* (1.58%). So, *N. gaditana* microalgae investigated in this study, representes a good raw material for the production of biofuels, due to their high lipid content, which may enhance the environmental cultivation possibilities without any competition with food crops.

On the other hand, the lipid level is less than what was found in the other works. Previous study done on *Nannochloropsis* sp. culture under various cultivation time and different photoperiod cycles (24/0, 18/06 and 12/12 h light/dark) showed a 31.3% lipid content (Wahidin et al., 2013). Similar results have been observed with *Nannochloropsis* sp. grown under nitrogen limitation, showing a 68.5% lipid content (Bondioli et al., 2012). This result can be explained by the culture method of microalgae, since the company that supplied the microalgae (*Nannochloropsis gaditana*) aimed at optimizing the production of biomass and not production of lipid. Massart et al. (2010) showed that increasing the growth leads to an oil level reduction.

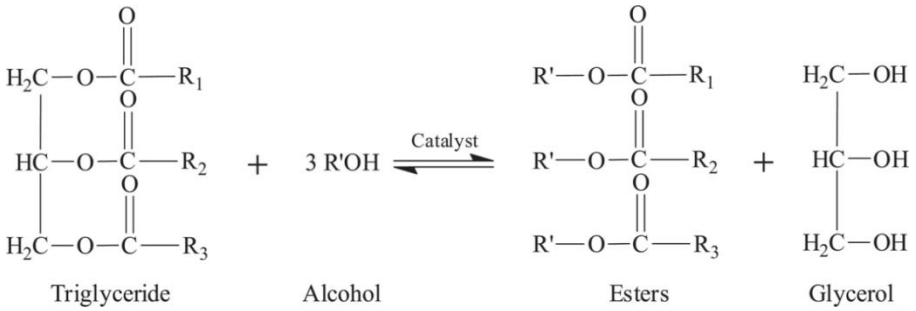

Figure 1. Transesterification reaction.

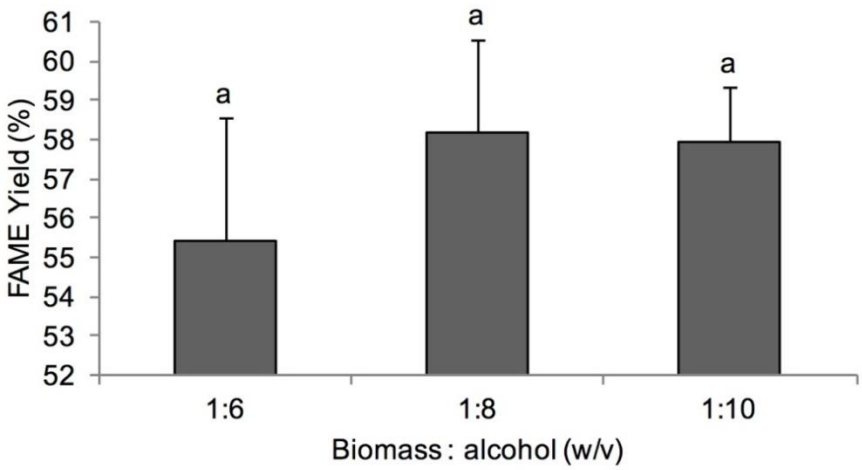

Figure 2. Influence of the biomass : alcohol ratio on biodiesel yield (at 90°C for 90 min using 60% wt. H_2SO_4). Averages with different indices are significantly different ($p < 0.05$).

Effect of alcohol volume

According to the majority of studies, this ratio appears to be the most important factor governing the speed of the transesterification reaction. The stoichiometry of the reaction involves the use of three moles of alcohol per one mole of triglyceride in order to obtain one mole of glycerol and 3 moles of fatty acid esters (Suganya and Renganathan, 2013).

However, the transesterification reaction is an equilibrium reaction in which a large excess of alcohol is required to promote the reaction in the direction of ester formation (Figure 1) (Refaat, 2009; El-Shimi et al., 2013; Meher et al., 2016).

The present study confirms the importance of using an excess methanol in transesterification process. The results which are presented in Figure 2, illustrate that the increase in the algae biomass: methanol ratio from 1:6 to 1:8 induces the increase in biodiesel yield from 55.4 ± 3.1% to 58.2 ± 2.4 %. Excess alcohol has a positive effect on biodiesel yield, but when the biomass: alcohol ratio is greater than 1:8, it has a negative impact (as observed) (Patil et al., 2012). In fact, the excess alcohol interferes with glycerin by increasing its solubility. When glycerin remains in solution, it helps drive the equilibrium to back to the left, reducing the ester yield (Figure 1) (Choo, 2004; Nautyal et al., 2014; Meher et al., 2016).

Similar results have been observed by several studies, Schwab et al. (1987) showed that to maximize FAME yields, a molar ratio of 1:6 should be used. They also mentioned that a molar ratio greater than 1:6, makes it more difficult to decant the glycerol and to separate the ester from it. Enciner et al. (2002) studied the trasesterification of *Cynara* oil by ethanol for a ratio of 1:3 to 1:15. The best results were obtained for molar ratios between 1:9 and 1:12. At a molar ratio of 1:15, the separation of glycerin becomes difficult. Schwab et al. (1987) reported that when glycerin remains in the reaction medium, it contributes to the shift of equilibrium towards the formation of triglycerides by lowering the yield of the ester.

Effect of catalyst concentration

In addition to the biomass: alcohol ratio, the concentration

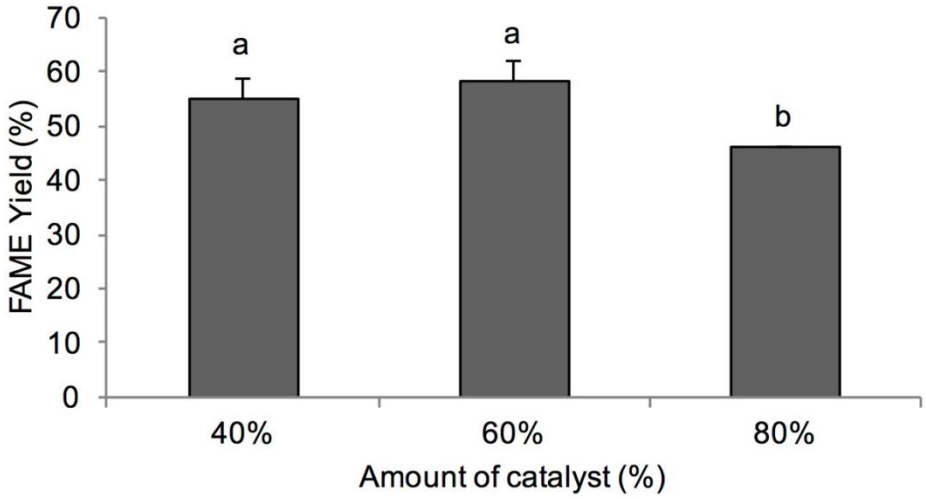

Figure 3. Influence of the catalyst concentration on biodiesel yield (at 90°C for 90 min using biomass to methanol ratio of 1:8). Averages with different indices are significantly different ($p < 0.05$).

of catalyst represents a very important variable in the conversion of the oil into FAME. The *in situ* transesterification process uses acid, basic or enzymatic catalysts. An acid catalyst (sulfuric acid) was chosen for this study because the acid-catalyzed reactions were found to be affective in converting the free fatty acids and the triglycerides into FAME (Ejikeme et al., 2010; Bradley et al., 2011).

In order to study the imparct of the concentration of H_2SO_4 on the FAME yield of *N. gaditana*, the concentration of the catalyst was varied from 40 to 80% (of dry algae biomass). From Figure 3, it can be observed that an increase in H_2SO_4 concentartion from 40 to 60% give an increase in FAME yield up to 58.2 ± 3.8%. Similar results was reported by Nautyal et al. (2014), they investigated the transesterification of *Spirulina platensis* using H_2SO_4 as the acid catalyst. As a result, the maximum production of FAME of 65.6% was obtained with 60% of catalyst concetration.

However, increasing the H_2SO_4 concentration from 40 to 60% induces a decrease in oil conversion into biodiesel (46.0 ± 0.1%). This result can be explained by the negative effect of high concentration in acid catalyst, which may lead to ether formation by alcohol dehydration and, the consequent high use of calcium oxide in the acid neutralization after production with its attendant high production cost and waste generation (Ejikeme et al., 2010).

Effect of reaction time

To study the effect of the reaction time on the conversion rate, three tests (30, 90 and 150 min) were carried out. In this experience, it was observed that when increasing the time reaction up to 90 min, the FAME yield increases from 55.2 ± 4 to 65.6 ± 2.1% (Figure 4). From this, it can be concluded that the biodiesel yield increase with the increase of the time reaction. This results are similar to those obtained by Freedman et al. (1984).

In this work, the method of direct or *in situ* transesterification for the production of FAME from *N. gaditana* oil was studied. A maximum biodiesel yield of 65.6% was arrived at as reported earlier by Shenbaga et al. (2012), who found FAME rates of 66.6 and 68.5% for *Dunaliella salina* and *Nannochloropsis* sp., respectively.

The direct transesterification reaction allows a more interesting production of biodiesel than the two-step transesterification which involves the extraction and conversion of the oil into FAME. The *in situ* transesterification makes it possible to gain more reaction time and also helps to avoid the potential loss of lipids during the extraction step (Johnson and Wen, 2009; Rekha et al., 2012).

Fatty acid profile and properties of microalgae oil and biodiesel

The FAME profile of *N. gaditana* is shown in Table 1. The most abundant fatty acid methyl ester was methyl palmitate, followed by methyl linoleate and methyl myristate, a similar results was reported by Afify et al. (2010). These FAMEs were reported to be common components in biodiesel. Advantageously, among these FAMEs, methyl palmitate whose quantity ranked first in this study was established as one of the biodiesel components that provide highest cetane response (Chattip et al., 2012).

The result shows that the biodiesel extracted of *N.*

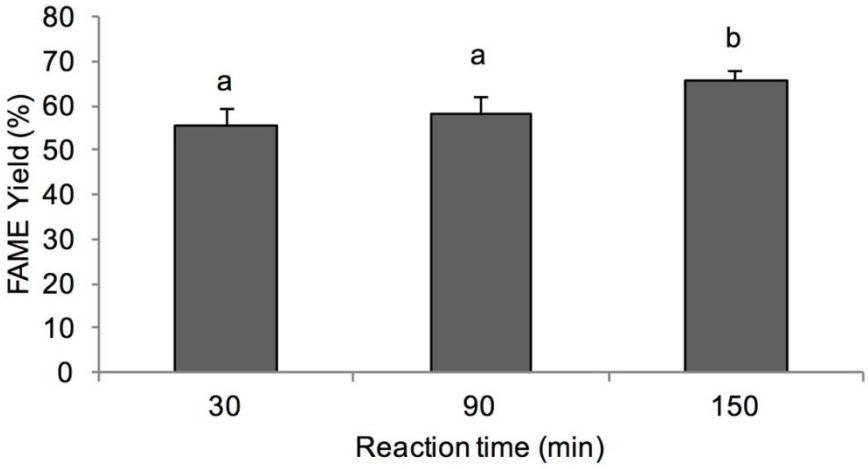

Figure 4. Influence of reaction time on biodiesel yield (at 90°C using biomass to methanol ratio of 1:8, 60% wt. H_2SO_4). Averages with different indices are significantly different ($p < 0.05$).

gaditana is composed of about 88.04% saturated fatty acid and about 11.96% unsaturated fatty acid, a similar trend was reported by Nautyal et al. (2014), who reported that the presence of highly saturated acids leads to increase in the stability of biodiesel.

Sarin et al. (2007) reported that the composition of the palm biodiesel was about 56.6% of unsaturated fatty acids and 43.4% of saturated fatty acids. Similarly, tallow biodiesel was reported to be composed of about 56.7% of unsaturated fatty acids and 42.8% of saturated fatty acids (Alcantara et al., 2000). Therefore, the higher percentage of saturated fatty acid in algae biodiesel makes it more stable as compared to tallow and palm biodiesel.

The average molecular weight of the oil and biodiesel extracted from *N. gaditana* revealed values of 851.10 and 286.02 g/mol, respectively. The same results were reported by El-Shimi et al. (2013), who found that the average molecular weight of the oil and biodiesel of *S. platensis* was 845.19 284 g/mol, respectively.

Cetane number of *N. gaditana* biodiesel was calculated to be 69.38, which is higher as compared to 60, for *S. platensis* (El-Shimi et al*.,* 2013), 45.8 for rapeseed biodiesel (Encinar et al., 2010) and also better than 38 for jatropha biodiesel (Sivaramakrishnan and Ravikumar, 2012). CN of biodiesel is generally higher than conventional diesel because it has longer fatty acids carbons and saturated molecules. The study of biodiesel CN has a high importance; since inadequate CN result in poor ignition quality, delay and excessive engine knock (Bamgboye and Hansen, 2008).

FT-IR analysis

More recently, FT-IR spectroscopy was used for the characterization of biodiesel (Meher et al., 2016). Generally, in the biodiesel samples, FT-IR spectra showed five important absorption bands. It can be observed that the C-H stretching absorbtion occurs at wavelength 2919.90 cm^{-1}, this peak appears strong in microalgae biodiesel samples as shown in Figure 5. Two alkanes peaks which is attributed to the bending absorbtion of methyl ($-CH_3$) and methylene ($-CH_2$) group appear at 1455.90 and 1361.25 cm^{-1}, respectively. Since biodiesel is mainly mono-alkyl ester, the intense C=O stretching band of methyl ester appears at 1739.26 cm^{-1}. One peak observed at 1167.70 cm^{-1} is due to stretching absorption of ester C-O. These results are in agreements with literature (Guil-Guerrero et al., 2004; Ching et al., 2011; Patil et al., 2012).

Ching et al. (2011) reported the same five peaks as *N. gaditana* after analysis of five microalgae biodiesel (*Nannochlororpsis oculata, Dunaliella tertiolecta, Chlorella vulgaris, Selenastrum capricornutum* and *Chlamydomonas reinhardtii)* by FT-IR.

Yadav et al. (2014) reported nine different absorption peaks after analysis of *Hydrodictyon reticulatum* (L) *Lagerheim* green algae biodiesel. Among them, the band at 1740 cm^{-1} which is associated with vibration of C=O shows ester groups, primarily from lipids and fatty acids (Coates, 2000).

Conclusion

The results of this study show that the optimal conditions for maximum FAME yield (65.6%) were determined as: biomass to methanol (w/v) ratio of 1:8, sulfuric acid concentration of about 60% (wt. wt^{-1}. oil), reaction time of 150 min and reaction temperature of 90°C.

The average mass molecular of microalgae oil was calculated to be 851.10 g/mol, reduced to 286.02 g/mol

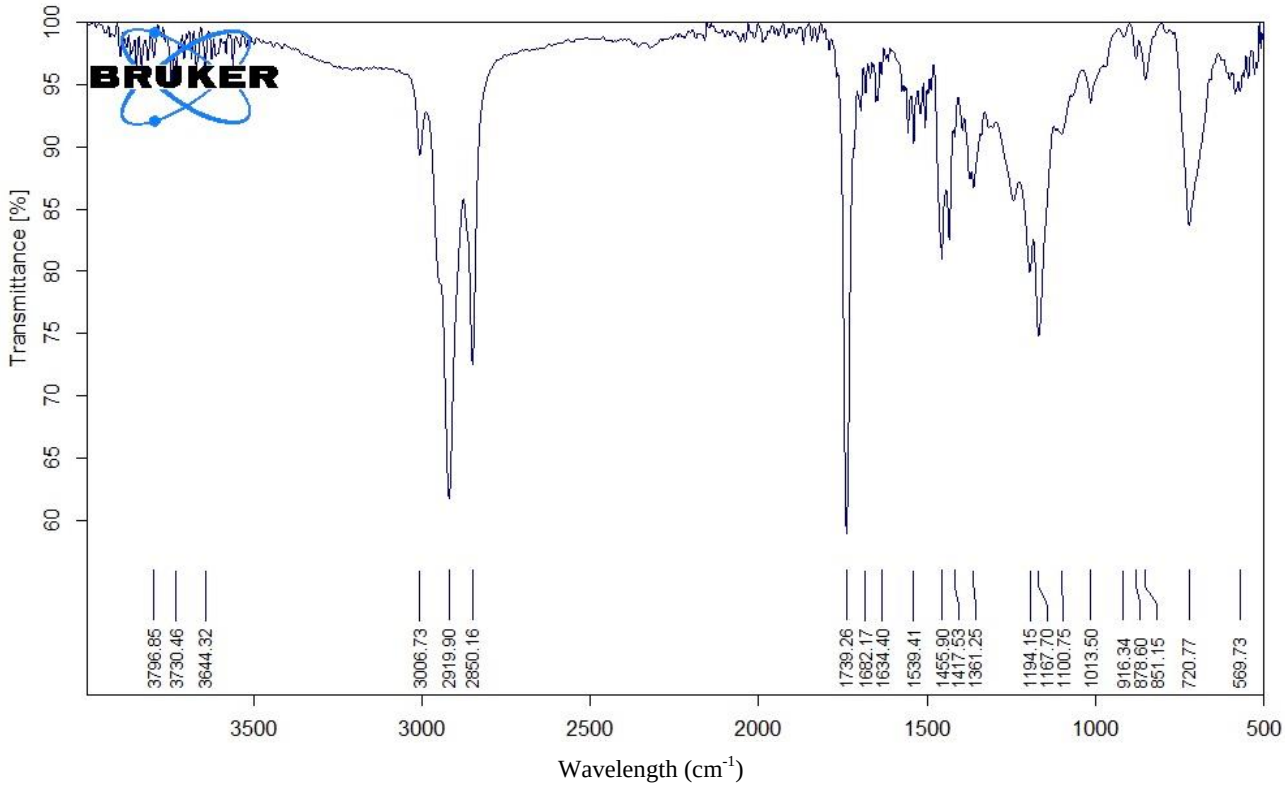

Figure 5. FT-IR analysis of FAME converted *N. gaditana* algal biomass.

for the production of FAME and the cetane number was 69.68, so *N. gaditana* microalgae investigated in this study, is proven to be suitable as raw materials for biodiesel production. Due to the FT-IR result and fatty acid profile, it is indicated that microalgae could produce high quality biodiesel and can be considered as potential feed stock for biodiesel production to fight the future energy crisis.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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