Production of green and renewable biodiesel from marine brown alga *Sargassum tenerrimum*

Abdul Majeed Khan*, Mariam Ameen & Noureen Fatima

Research Laboratory of Bioenergy (RLB), Department of Chemistry, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, University Road, Karachi-75300, Pakistan

[E.Mail: dr.abdulmajeedkhan@fuuast.edu.pk]

Received 05 February 2016 ; revised 17 November 2016

The present research work demonstrates the production of biodiesel from the oily content of marine macroalga *Sargassum tenerrimum* via mechanical stirring and microwave irradiations. The algal oil as well as direct algal biomass were subjected to methanolysis and ethanolysis using Na metal, NaOH and H$_2$SO$_4$ as catalysts. Mechanical stirring was found to be relatively slow but more feasible method for transesterification while microwave irradiation was observed to be too fast method with certain limitations like vigorous bumping that would be uncontrollable on large scale. Na metal was the most reactive catalyst that produced FAME (82%) and FAEE (80%) by mechanical stirring at room temperature whereas it produced FAME (88%) and FAEE (85%) by microwave heating within 1-5 minutes. Na metal was found to be very reactive, NaOH was the moderate while H$_2$SO$_4$ was the slowest catalyst for transesterification. Methanol was found to be more reactive due to its smaller size as compared to ethanol. Algal oil produced significant amount of biodiesel as compared to the algal biomass due to maximum interaction of reactants with oil. Biodiesel production was confirmed by TLC examination and by comparing the fuel properties of biodiesel with the ASTM standard limits of biodiesel.

**Keywords:** *Sargassum tenerrimum*, Oily contents, Methanolysis, Ethanolysis, Mechanical stirring and Microwave irradiations

**Introduction**

The current energy demands are mainly relied on fossil fuels that are toxic to the environment and non-renewable in nature$^{1,2}$. In this scinerio, renewable and environment friendly alternatives of energy are required to replace the fossil fuels$^{3-5}$. Biodiesel is the green fuel and best substitute of petrodiesel having low emission profile of toxic gases like SO$_X$, CO$_2$ and CO$^{6-8}$. It is derived from the renewable biological sources such as animal fats, vegetable oils, macroalgae, microalgae, waste cooking oil and oil enriched plants such as castor, jatropha, pongamia and karanja etc$^{6,9-11}$. Algae are photosynthetic organisms that grow rapidly and can be harvested on large scale$^{6,12}$. Direct transesterification of algal biomass to biodiesel has also been reported that saves the solvent and reduces the steps$^{13}$. Catalyzed reactions are fast at low temperature but produce the soap as byproduct. On the other hand, acid catalyzed reactions are slow, reversible and they do not produce soap$^{15,16}$. Microwave irradiations are effective to produce biodiesel that reduce the reaction time, save energy and produce high yield of the product$^{17}$.

This research article is based on the investigation of marine brown macroalga *Sargassum tenerrimum* for the production of biodiesel. The detailed study of fatty acid composition of *S. tenerrimum* is already reported$^{18}$. Some of the species from genus *Sargasso* including *Sargassum cymosum* have been reported for the production of biodiesel$^{19}$. **Materials and Methods**

*Sargassum tenerrimum*, marine brown alga (Fig. 1) was collected during 2010 from the Bulleji coast, South Karachi, Pakistan. The alga was
identified by Prof. Dr. Mustafa Shameel (late), Department of Botany, University of Karachi, Pakistan. The alga was dried under shadow for seven days and then under sunlight for six days. The alga was grinded into powder by Black and Decker FX35OB chopper. The powdered alga (7 kg) was soaked in \(n\)-hexane : chloroform (1:1; 9 liters) for a week. The \(n\)-hexane extract was separated by decantation and the same process was repeated for three times. The solvent phase was evaporated from the extract by rotavapor under reduced pressure hence the concentrated dark green crude oil was obtained. The crude oil was mixed with charcoal (50 g) and kept for 24 hours that removed the pigments from oil. In addition, the oil was passed through the column chromatography to purify it using pet-ether as mobile phase and silica gel (mesh size 100-200) as stationary phase. The oil obtained from dried algal biomass (7 kg) through extraction using \(n\)-hexane : chloroform (1:1) was found to be 3%.

The transesterification of the algal oil and algal biomass was performed by mechanical stirring as well as by microwave irradiation using Na metal, NaOH and \(\text{H}_2\text{SO}_4\) as catalysts to yield the fatty acid methyl ester (FAME) and fatty acid ethyl ester (FAEE).

The algal oil (2 g) and Na metal (0.3 g) dissolved in methanol or ethanol (10 ml) were taken in an Erlenmeyer flask and stirred on the hot plate stirrer (Lab Tech * Daihan Labtech Co., Ltd) at room temperature for 20 minutes. Distilled water was added in the reaction mixture and shaken well then kept for an hour to settle down. Two distinct layers, the upper biodiesel and the lower water/glycerol layers were formed which were separated by separating funnel. The solvents were allowed to evaporate from both the layers to obtain the desired products i.e. biodiesel and glycerol. Likewise, the algal oil was treated with NaOH (0.3 g) at room temperature for 3 hours and at 60 °C for 1 hour. Similarly, the reaction was performed in the presence of conc. \(\text{H}_2\text{SO}_4\) (0.2 ml) at room temperature for 24 hours and at 60 °C for 6 hours. During the reaction, the product was taken at different intervals of time and the progress of biodiesel production by TLC examination was checked. The same reactions were conducted for the synthesis of biodiesel directly from algal biomass (10 g) instead of using algal oil.

Algal oil (2 g) and Na metal (0.3 g) dissolved in methanol or ethanol (10 ml) were taken in Erlenmeyer flasks and refluxed in the modified microwave oven (Model-DW131HP) at 900 Watt output power, \(2.45 \times 10^9\) Hertz frequency and 60 °C for 5 minutes. The modified microwave oven was fitted with the reflux condenser connected with the chiller (Buchi, Germany). After completion of the reaction, the reaction mixtures were separated through the same method as used for mechanical stirring method to yield the FAME and FAEE. The reactions were also performed in the presence of NaOH (0.3 g) and conc. \(\text{H}_2\text{SO}_4\) (0.2 ml) as catalysts. A set of experiments were also performed using algal biomass (10 g) instead of algal oil to produce biodiesel. The desired products were separated from the reaction mixture through the proper workup as for mechanical stirring.
The concentrated biodiesel so produced by different reactions was passed through the column chromatography using silica gel (100-200 mesh size) and n-hexane as mobile phase to remove the pigments and other impurities. After purification, the % yields of FAME and FAEE were calculated using the formula:

\[
\text{Biodiesel yield (\%)} = \frac{\text{Biodiesel produced}}{\text{Oil used}} \times 100
\]

The TLC examination of FAME and FAEE was conducted to confirm the biodiesel production. The TLC examination of oil, FAME and FAEE was performed using silica gel coated aluminium Merck grade cards as stationary phase and pet-ether : chloroform : toluene (7:2:1) as mobile phase. The spots of biodiesel were visualized by iodine vapors. Furthermore, density, kinematics viscosity, acid value, cloud point and pour point of both FAME and FAEE were determined and results were compared with algal oil and the ASTM standard limits for biodiesel.

**Results**

The percentage yield of oily contents of *S. tenerrimum* was calculated and found to be 3% with respect to the dry weight of alga. Pigments present in oil were removed by charcoaling and it was further purified by column chromatography that resulted in the pure yellow oil. TLC of the algal oil confirmed the presence of some organic compounds. Iodine vapors showed the dark brown spots of fatty acids or triglycerides whereas four vanillin active spots showed the presence of steroids, terpenoids and other organic compounds. In addition, the GC spectrum of *S. tenerrimum* showed many peaks in the range of 34 to 72 minutes retention time that detected the presence of a number of fatty acids in the algal oil (Fig. 2).

Furthermore, density, kinematics viscosity, acid value, cloud point and pour point of the algal oil were also determined but the values were found higher than the ASTM standard limits of biodiesel. Therefore, the algal oil must be converted to its alkyl esters to be used as diesel engine fuel.

The mechanical stirring of algal oil was carried out using Na metal, NaOH and conc. H₂SO₄. Na metal was found to be very reactive catalyst that produced biodiesel (FAME: 88% and FAEE: 85%) within 5 minutes at room temperature. NaOH was found to be the next reactive catalyst that converted the oil into biodiesel (FAME: 87% and FAEE: 85%) within 60 minutes at 60 °C with continuous mechanical stirring. The reactivity of conc. H₂SO₄ was observed to be slow and the maximum biodiesel (FAME: 78% and FAEE: 73%) was produced at 60 °C after 6 hours (Fig. 3, Table 1-2).

![Fig. 2- GC spectrum of *S. tenerrimum* oil.](image-url)
The microwave irradiated transesterification of algal oil produced the biodiesel very fast due to the high speed of electromagnetic irradiations. In the modified microwave oven, both the NaOH and Na metal produced significant amount of biodiesel in 5 minutes (Table 1-2).

The reaction in the presence of Na metal was observed to be very rapid and it started bumping after 3 minutes that could damage the radiation source. NaOH was the most suitable catalyst for the modified microwave oven that produced the high yield of biodiesel within 5 minutes. On the other hand, conc. H₂SO₄ did not produce the product during 5 minutes in the modified microwave oven. However, the literature showed that 20 minutes are required to complete biodiesel synthesis through microwave irradiations using conc. H₂SO₄ as catalyst\(^{20}\) (Fig. 3, Table 1-2).

Soap and glycerol were formed as by-products during the reaction which were catalyzed by Na metal and NaOH catalysts. The by-products were separated by washing with hot water. On the other hand, the reaction catalyzed by conc. H₂SO₄ did not form soap however it gave reversible reaction and its reactivity was found to be slow as compared to Na metal and NaOH. In addition, H₂SO₄ showed 19% and 48% conversion of algal oil to biodiesel after 12 and 24 hours respectively at room temperature while ethanol did not produce biodiesel at room temperature. Moreover, low biodiesel yield was observed in case of FAEE as compared to FAME using each catalyst because methanol is more reactive than ethanol. The transesrterification using algal biomass did not produce significant yield of biodiesel because the reactants were not in the direct contact with the algal oil (Fig. 3, Table 1-2). All the reactions were performed in the anhydrous conditions using silica gel as moisture absorbent. In addition, the samples were placed in the desiccators to avoid the contact of moisture with the reactants. Under hydrous conditions, the biodiesel is hydrolyzed back to free fatty acids therefore, the conditions were made completely anhydrous (Fig. 4).
Table 2- Yields (%) of FAME and FAEE produced by different methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Catalyst</th>
<th>Mechanical stirring</th>
<th>Microwave irradiations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time (min.)</td>
<td>Temp. (°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FAME</td>
<td>FAEE</td>
</tr>
<tr>
<td>Oil</td>
<td>Na metal</td>
<td>20</td>
<td>31</td>
</tr>
<tr>
<td>Oil</td>
<td>NaOH</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Oil</td>
<td>H₂SO₄</td>
<td>360</td>
<td>60</td>
</tr>
<tr>
<td>Dry biomass</td>
<td>Na metal</td>
<td>20</td>
<td>31</td>
</tr>
<tr>
<td>Dry biomass</td>
<td>NaOH</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Dry biomass</td>
<td>H₂SO₄</td>
<td>360</td>
<td>60</td>
</tr>
</tbody>
</table>

Fig. 4-Hydrolysis of biodiesel to free fatty acids.

The biodiesels obtained by different methods were purified by the column chromatography. The comparative TLC of algal oil, FAME and FAEE confirmed the production of biodiesel. The Rf values of FAME and FAEE were observed to be 0.30 and 0.29 respectively (Fig. 5). The density, kinematics viscosity, acid value, cloud point and pour point of FAME and FAEE were found to be within the ASTM standard limits for biodiesel\textsuperscript{21,22} (Table 3).

Fig. 5-TLC examination of oil, FAME and FAEE.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Oil</th>
<th>FAME</th>
<th>FAEE</th>
<th>ASTM Standard limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>g / cm(^3)</td>
<td>1.10 ± 0.5</td>
<td>0.87 ± 0.2</td>
<td>0.88 ± 0.1</td>
<td>0.875 to 0.9</td>
</tr>
<tr>
<td>Kinematics viscosity</td>
<td>mm(^2) / sec</td>
<td>19.60 ± 0.4</td>
<td>4.25 ± 0.1</td>
<td>4.68 ± 0.3</td>
<td>1.9 to 6.0</td>
</tr>
<tr>
<td>Acid value</td>
<td>mg KOH / g sample</td>
<td>6.31 ± 0.1</td>
<td>0.65 ± 0.3</td>
<td>0.70 ± 0.5</td>
<td>0.8 max</td>
</tr>
<tr>
<td>Pour point</td>
<td>°C</td>
<td>0 ± 1</td>
<td>-1 ± 1</td>
<td>-3 ± 1</td>
<td>-15 to 16</td>
</tr>
<tr>
<td>Cloud point</td>
<td>°C</td>
<td>2 ± 1</td>
<td>0 ± 1</td>
<td>1 ± 1</td>
<td>-3 to 12</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation.

Discussion
The current global challenges like global warming, energy crisis, environmental pollution, biodiversity, economy and many others are the driving factors which stimulated us to investigate the alternative renewable sources of green energy that have a potential to address these challenges. For this purpose, the present research was focused on the production of biodiesel from marine macroalga particularly *Sargassum tenerrimum* using sodium metal, NaOH and H₂SO₄ as active catalysts for the transesterification of triglycerides of the alga. The study showed that all three catalysts are active to produce biodiesel. However, sodium metal was found to be more reactive than NaOH which in turn found to be more reactive than H₂SO₄. All the catalysts possessing some
advantages and disadvantages i.e. sodium metal and sodium hydroxide converted the free fatty acids to soap along with the production of biodiesel therefore they reduced the yield of desired product as well as resulted in the separation problems. On the other hand, H₂SO₄ produced the lower yield of biodiesel because under acidic condition the ester was hydrolyzed back to the carboxylic acids. Briefly, sodium metal was found to be best catalyst for biodiesel production.

Furthermore, conventional heating was compared with the microwave irradiations. The microwave irradiations are highly energetic and produced the desired product within shortest time as compare to conventional heating. In addition, the algal extracted oil and the dry mass of the same alga were also compared to check the feasibility of the methodology. It was observed that the reaction of the oil with the alcohol is more favorable than the reaction with dry algal mass. The dry algal mass produced low yield of the product because of very low interaction of alcohol with the oily content.

This research will play a vital role for the green environment, energy crisis, public health, biodiversity, decline in agriculture, oil based international political stress and natural disasters like heavy rain, floods and melting of glaciers.

Conclusions

The algal oil was transesterified using Na metal, NaOH and conc. H₂SO₄ catalysts by mechanical stirring as well as microwave irradiations to produce biodiesel. During biodiesel synthesis, fast mechanical stirring is necessary for the rapid mixing of different phases of reactants. High intensity electromagnetic radiations i.e. microwave irradiation was also very effective in transesterification reaction that do not need mechanical stirring. Na metal was found to be most reactive, NaOH mild reactive while H₂SO₄ was least reactive catalyst. Na metal and NaOH produced soap as by-product which is the major drawback of these catalysts. On the other hand, H₂SO₄ is slow catalyst and it gave reversible reaction that reduced the overall yield of the product. Ethanol was found to be slightly less reactive as compared to methanol due to its larger size. The fuel properties of methyl ester are better for diesel engine as compared to ethyl ester. Shorter the alkyl chain, less will be the viscosity of the biodiesel and favorable will be the combustion of the fuel in the engine. Algal oil after the extraction was found to be more favorable for biodiesel production in contrast to the algal biomass. The reactions were performed under completely anhydrous conditions to avoid reversibility of reaction. The biodiesel production was confirmed by the TLC examination. In addition, the fuel properties like density, kinematics viscosity, acid value, cloud point and pour point of biodiesel were found within the ASTM standard limits for biodiesel which also gave strong evidence of the production of biodiesel. This research will be more fruitful to mitigate the environmental pollution and to reduce the global warming and energy crisis around the globe.

Acknowledgments

Authors are greatly thankful to Prof. Dr. Mustafa Shameel (late), Department of Botany, University of Karachi for the identification of alga.

References

11. Khan, A. M., Fatima, N., Hussain, M. S. and Yasmeen, K., Biodiesel production from green seaweed Ulva fasciata catalyzed by novel waste


