Article

Analysis and Characterization of Algal Oil by Different Chromatographic Techniques for the High Production of Biodiesel from *Scenedesmus dimorphus*

Gulab Chand Shah *, Alkesh Patidar, Vikash Urkude, Anil Hurmale, Sudheer Choudhary, Mahavir Yadav, Archana Tiwari

School of Biotechnology, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal, Madhya Pradesh (University of Technology of Madhya Pradesh), Airport Bypass Road, Gandhi Nagar, Bhopal-462 033, India

* Author to whom correspondence should be addressed; E-Mail: gulab777@gmail.com; Tel.: 0755-2678803; Fax: 0755-2742002-3.

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**Abstract:** Biodiesel is an alternative fuel for conventional diesel that is made from natural plant oils, animal fats, and waste cooking oils. Algae are the fastest-growing plants on the earth, and very important as a biomass source. Microalgae have been identified as a potential biodiesel feedstock due to their high lipid productivity and the process conditions are milder than those required for pyrolysis and prevent the formation of by-products. This study demonstrates the culturing of algal strain on MBM, CHU13 media and production of algal biodiesel from *Scenedesmus dimorphus*, and discusses the economics of producing oil from algae grown in open ponds by Soxhlet, ultrasonic wave, and expeller method. In addition, the algal oil was analyzed by TLC and paper chromatography. Algae will some day be competitive as a source for biofuel. Algae can be grown almost anywhere, even on sewage or salt water, and does not require fertile land, and processing requires less energy than that provided by the algae. Algae can be a replacement for oil based fuels, which is more effective and has no disadvantages. About 50% of algal oil converted to biodiesel by transesterification process. This microalgal oil can be used to make biofuels for bus, and other vehicles.

**Keywords:** microalgae; *Scenedesmus dimorphus*; biofuels; lipid; biomass; glycerol; transesterification; chromatography.
1. Introduction

A constant rising worldwide demand of motor and power generation fuels, together with environmental concerns in terms of green house gases (GHG), has motivated the scientists and technologists to think about various alternate sources of energy (Singh et al., 2010). With the increasing amount of waste originating from human activities comes the negative impact on the environment and in particular the water quality. Waste streams, which are rich in carbon, nitrogen and other minerals, have potential for use as a substrate for microalgae cultivation (Hammouda et al., 1995; Hoffmann, 1998). Biodiesel is derived from the transesterification of mono-, di- and tri-acylglycerides (TAGs) and the esterification of free fatty acids (FFAs) that occur naturally in biological lipids, such as animal fats and plant oils. As a result, biodiesel has the potential to be a carbon neutral fuel (Freedman et al., 1984; Lopez et al., 2005; Ma and Hanna, 1999).

Although industrial-scale facilities for biodiesel production from microalgae have not been built, there has been substantial research performed on the feasibility, design and requirements for such a production system. A near-complete design for a large (400 ha) production system to produce biodiesel from algae is in (Regan et al., 1983), as well as recommendations on exactly where in Australia such facilities could be situated, whilst (Benemann et al., 1996) contains additional information on algal production, including economic considerations and identifies several additional pieces of equipment necessary for production not outlined in (Regan et al., 1983).

It would be valuable to be able to extract and convert triglycerides in microalgae into biodiesel in a single step, bypassing the use of large quantities of organic solvents. Such in situ or direct transesterification approaches have been used as an analytical technique to prepare FAMEs for the determination of the fatty acid composition of lipid containing tissues (Lepage and Roy, 1984; Park and Goins, 1994; Rodríguez-Ruiz et al., 1998).

Higher biomass productivity and lower production costs will also encourage production in the tropics. Therefore, biofuels have the potential to provide opportunities for economic development and improved energy access for developing countries. However, the negative impacts of increased global demand for biofuels are of increasing concern, and include direct and indirect land use change, competition with food production, and land tenure conflicts (Doornbosch and Steenblik, 2007; Ivanic and Martin, 2008; Renewable Fuels Agency, 2008; Searchinger et al., 2008; Semino et al., 2007; Sylvester-Bradley, 2008; Wiggins et al., 2008).

This paper studies the culturing of algal strain on MBM, CHU13 media and production of algal biodiesel from Scenedesmus dimorphus, and discusses the economics of producing oil from algae grown in open ponds by Soxhlet, ultrasonic wave, and expeller method. In addition, the algal oil was analyzed by TLC and paper chromatography.
2. Materials and Methods

2.1. Materials

The proposed study was done at School of Biotechnology, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal, Madhya Pradesh. All the chemicals and glasswares used in the proposed study were procured from Himedia and VK traders, respectively. All the techniques and protocols used in the proposed study were standardized according to the literature available.

Algae samples were collected from various places, such as Upper lake, Colar dam, pond near bhanpur dumping sites, and Narmada hosangabad.

2.2. Methods

2.2.1. Identification of suitable strain (Carlos et al., 2010)

**A. Media for Scandasmus dimorphus algae growth**

There were two types of media used in desire algal culture. Elemental compositions are given in Tables 1 & 2.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chemical</th>
<th>Quantity (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(NH$_2$)$_2$CO</td>
<td>1800</td>
</tr>
<tr>
<td>2</td>
<td>KH$_2$PO$_4$</td>
<td>1250</td>
</tr>
<tr>
<td>3</td>
<td>MgSO$_4$.7H$_2$O</td>
<td>1000</td>
</tr>
<tr>
<td>4</td>
<td>EDTA</td>
<td>500</td>
</tr>
<tr>
<td>5</td>
<td>H$_3$BO$_4$</td>
<td>114.2</td>
</tr>
<tr>
<td>6</td>
<td>CaCl$_2$.2H$_2$O</td>
<td>111</td>
</tr>
<tr>
<td>7</td>
<td>FeSO$_4$.7H$_2$O</td>
<td>49.8</td>
</tr>
<tr>
<td>8</td>
<td>ZnSO$_4$.7H$_2$O</td>
<td>88.2</td>
</tr>
<tr>
<td>9</td>
<td>MnCl$_2$.4H$_2$O</td>
<td>14.2</td>
</tr>
<tr>
<td>10</td>
<td>CuSO$_4$.5H$_2$O</td>
<td>15.7</td>
</tr>
<tr>
<td>11</td>
<td>Co(NO$_3$)$_2$.6H$_2$O</td>
<td>4.9</td>
</tr>
</tbody>
</table>

**B. Media preparation**

Take above medium and make it 100 mL, autoclave it at 121 °C, 15 lbs pressure, for 15 min. Inoculate algal sample into four different conical flasks (100 mL). Incubate it at normal RT, for 24 h, Observe growth into different conical flasks. Select one algae containing flask, which have maximum growth, transfer it into 1000 mL media containing flask, monitor growth of algae (day/day). After 10 - 15 days, algal strain has to be used to further processing.
Table 2. Composition of modified CHU 13 medium

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Composition</th>
<th>Stock Media 20X (g/L)</th>
<th>Working media 1X (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KNO₃</td>
<td>8</td>
<td>400</td>
</tr>
<tr>
<td>2</td>
<td>K₂HPO₄</td>
<td>1.6</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>MgSO₄·7H₂O</td>
<td>4</td>
<td>200</td>
</tr>
<tr>
<td>4</td>
<td>CaCl₂</td>
<td>2.14</td>
<td>107</td>
</tr>
<tr>
<td>5</td>
<td>Ferric citrate</td>
<td>0.4</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>Citric acid</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>CoCl₂·2H₂O</td>
<td>2.14</td>
<td>107</td>
</tr>
<tr>
<td>8</td>
<td>H₃BO₃</td>
<td>114.4</td>
<td>5.72</td>
</tr>
<tr>
<td>9</td>
<td>MnCl₂·4H₂O</td>
<td>73.4</td>
<td>3.67</td>
</tr>
<tr>
<td>10</td>
<td>ZnSO₄·7H₂O</td>
<td>8.8</td>
<td>0.44</td>
</tr>
<tr>
<td>11</td>
<td>CuSO₄·5H₂O</td>
<td>3.2</td>
<td>0.16</td>
</tr>
<tr>
<td>12</td>
<td>NaMoO₄</td>
<td>1.68</td>
<td>0.084</td>
</tr>
<tr>
<td>13</td>
<td>0.72N H₂SO₄</td>
<td>1 drop</td>
<td></td>
</tr>
</tbody>
</table>

2.2.2. Algae Harvesting  (Carlos et al., 2010; Shelef et al., 1984)

**A. Micro-screening**

Algae with media in open pond was carefully taken on (250 µm, 500 µm) size sieves, filter and discard excess water, dry it in shade.

**B. Centrifugation**

Medium containing algae from open pond, if algal concentration is very low, take 30 mL centrifuge tube, transfer medium containing algae into the tube. Centrifuge it with 4000 rpm for 5 min at room temperature. Discard supernatant and keep pellets for further oil processing.

2.2.3. Extraction of oil from algae


Take 500 g of shade dried algae. Transfer it into expeller machine for extraction of algal oil. Run the expeller machine. After some time collect algal oil from machine, make it for further transesterification process for biodiesel production.

**B. Soxhlet extraction** (Carlos et al., 2010)

Take 100 g dried powder of algae, keep it into Soxhlet apparatus. Add 100 mL hexane solvent, to rapture cell wall of algae. Run the Soxhlet (containing algae and hexane), after 20 h algal oil was collected from round bottom flask. The algal oil was used for biodiesel production.

**C. Ultrasonic-assisted extraction** (http://www.oilgae.com/algae/oil/extract/mec/mec.html)
Take 50 g of dried algae and add 100 mL of ether in 250 mL beaker, provide ultrasonic wave for 30 min, where ultrasonic waves are used to create cavitation bubbles in a solvent material. Ultrasonic wave also works as cell wall rupture of algae. Filter it with sieves, manually press algae to extract algal oil. The algal oil was used to further transesterification process.

### 2.2.4. Lipid analysis

**A. Thin layer chromatography** (kumara et al., 2011)

Silica paper was used as the template, mark the plates with a sharp pencil, and line the chamber with chromatography paper. Prepare 202 mL of solvent system (Hexane : Ether : Acetic acid, 60 : 40 : 1) in a 3000 mL chromatographic chamber. Mix and pour 202 mL into the chamber. Cover and let the chamber saturate while loading the plates. With a 10 μL capillary pipette, spot 1 - 2 μL of phospholipids standard onto the TLC paper. Make sure the spot remains smaller than 4 mm in diameter. Move on to the other standards. After the spots have dried, repeat loading each standard until you have loaded approx. 10 μL each. Also, load 10 μL of your lipid extract on one spot, and then the remainder of the extract as a line (i.e., a series of spots). Let dry the spots. Make sure that the loading area is above the solvent. Place the plates in the chamber to develop. Immediately close the cover and let run for approximately 60 min, until the solvent front has reached the upper line. Remove the plate and leave to dry in the rack in the fume hood. Discard the solvent in the waste container provided, remove the chromatography paper and leave in the chamber. Leave the chamber in the fume hood to dry. Now place the plate in the iodine tank in the fume hood. You will see the lipids as yellow spots after about 5 min or so. Mark the edges of the spots with a pencil.

**B. Paper chromatography** (Amsterdam et al., 1985)

Take the Whatman No. 1 chromatography paper of appropriate size, place it on a rough paper with the help of pencil and scale draw a line leaving 1.5 cm from the bottom. Now on the line mark seven spots leaving 1.5 cm on either side of the edges. Now measure the distance between the spots carefully draw three small circles touching the line, below the line under each circle write the name of the standard. Samples were loaded in center of the paper. With the help of capillary tube apply standard and the sample give a feather touch and see that the solute do not spread below the line. Now fold the paper in the form of a cylinder and staple at three different positions with the help of stapler. While stapling it, be careful and check that the two ends of the paper are equal and the spots are present outside the circle and there is a gap between the two edges.

### 2.2.5. Transesterification (Sree et al., 2009)

Algal oil will be highly viscous, one of the most common methods which are used to reduce oil viscosity in the algae oil is called transesterification. It involves chemical conversion of the oil into its
corresponding fatty ester. The 100 mL algal oil was kept in conical flask. Add 5 mL of 0.5 M KOH, and then add 70 mL methanol. Heat it with 70 °C on heating mental and after 2 h biodiesel was collected.

3. Results and Discussion

3.1. Sample Collection

Algal sample ware collected from different places of Bhopal region (Fig. 1).

![Figure 1. Different sample collection.](image)

3.2. Identification of Suitable Strain

The algal species *Scandasmus dimorphus* was grown on MBM medium and CHU13 medium (Fig. 2).

![Figure 2. Culture of different algal sample.](image)
The algal strain was obtained from Hosangabad sample, and grown in 500 mL MBM solution (Fig 3).

Figure 3. Media containing algal strain.

3.3. Algae Harvesting

A. Micro-screening
Algae harvesting was done after algal culturing by using (250 µm, 500 µm) sieves (Fig. 4).

Figure 4. Algae harvesting by sieves.
**B. Centrifugation**

In case of low growth rate of desire algal strain, centrifuge was used for algae harvesting (Fig. 5).

![Figure 5. Algal pallets are shown in the bottom.](image)

Take pallets and shaded dry, and also using lyophilizer for drying algae and then make it in powder form.

**3.4. Algal Oil Extraction**

**A. Ultrasonic-assisted extraction**

The ultrasonicator was used for algal oil extraction, and upper layer are algal oil, which is shown in the Fig. 6.

![Figure 6. Ultrasonic-assisted extraction.](image)

**B. Soxhlet method for oil extraction**

The Soxhlet apparatus was used for the oil extraction from algae, which is shown in Fig. 7.
C. Expeller press method for oil extraction

The expeller machine was used for the oil extraction from algae, which is shown in Fig. 8.

The oil extracted by different methods is shown in Table 3 and Fig. 9.

Table 3. Oil from different techniques

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Quantities of algae (g)</th>
<th>Quantities of oil by different method (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Soxhlet</td>
</tr>
<tr>
<td>01</td>
<td>500</td>
<td>125</td>
</tr>
<tr>
<td>02</td>
<td>250</td>
<td>60</td>
</tr>
<tr>
<td>03</td>
<td>100</td>
<td>20</td>
</tr>
</tbody>
</table>
3.5. Chromatographic Technique

A. Thin layer Chromatography

The thin layer chromatogram is shown in Fig. 10, and the RF values are given in Table 4.

![Image of thin layer chromatogram]

**Figure 10.** Thin layer chromatogram of different oil samples.

RF factor = Distance traveled by solute (cm)/Distance traveled by the solvent (cm)

A. RF factor of oil which was extracted by expeller method = 10/10.7 = 0.93
B. RF factor of oil which was extracted by Soxhlet method = 9.2/10.7 = 0.85
C. RF factor of oil which was extracted by Utrasonicator method = 5/10.7 = 0.46
D. RF factor of jatropha crude oil = 9.7/10.7 = 0.90
E. RF factor of jatropha biodiesel = 9.2/10.7 = 0.85
F. RF factor of karanja crude oil = 9.7/10.7 = 0.90
G. RF factor of karanja biodiesel = 10.2/10.7 = 0.95
Table 4. RF values of different oil samples using TLC

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of methods/sample</th>
<th>Distance travel by solute (cm)</th>
<th>Distance travel by solvent (cm)</th>
<th>RF value</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Expeller</td>
<td>10</td>
<td>10.7</td>
<td>0.93</td>
</tr>
<tr>
<td>02</td>
<td>Soxhlet</td>
<td>9.2</td>
<td>10.7</td>
<td>0.85</td>
</tr>
<tr>
<td>03</td>
<td>Ultrasonicator</td>
<td>5</td>
<td>10.7</td>
<td>0.46</td>
</tr>
<tr>
<td>04</td>
<td>Jatropha crude oil</td>
<td>9.7</td>
<td>10.7</td>
<td>0.90</td>
</tr>
<tr>
<td>05</td>
<td>Jatropha biodiesel</td>
<td>9.2</td>
<td>10.7</td>
<td>0.85</td>
</tr>
<tr>
<td>06</td>
<td>Karanja crude oil</td>
<td>9.7</td>
<td>10.7</td>
<td>0.90</td>
</tr>
<tr>
<td>07</td>
<td>Karanja biodiesel</td>
<td>10.2</td>
<td>10.7</td>
<td>0.95</td>
</tr>
</tbody>
</table>

B. Paper chromatography technique

The paper chromatogram is shown in Fig. 11, and the RF values are given in Table 5.

RF factor = Distance traveled by solute (cm)/Distance traveled by the solvent (cm)

A. RF factor of oil which was extracted by Soxhlet method = 13.7/18.7 = 0.73
B. RF factor of oil which was extracted by expeller method = 13.5/18.7 = 0.72
C. RF factor of oil which was extracted by Ultrasonicator method = 13.3/18.7 = 0.71
D. RF factor of jatropha crude oil = 16/18.7 = 0.85
E. RF factor of jatropha biodiesel = 15.7/18.7 = 0.83
F. RF factor of karanja crude oil = 15.8/18.7 = 0.84
G. RF factor of karanja biodiesel = 15.6/18.7 = 0.83

Figure 11. Paper chromatogram of different oil samples.
Table 5. RF values of different oil samples using paper chromatography

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of method</th>
<th>Distance travel by solute (cm)</th>
<th>Distance travel by solvent (cm)</th>
<th>RF value</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Soxhlet</td>
<td>13.7</td>
<td>18.7</td>
<td>0.73</td>
</tr>
<tr>
<td>02</td>
<td>Expeller</td>
<td>13.5</td>
<td>18.7</td>
<td>0.72</td>
</tr>
<tr>
<td>03</td>
<td>Ultrasonicator</td>
<td>13.3</td>
<td>18.7</td>
<td>0.71</td>
</tr>
<tr>
<td>04</td>
<td>Jatropha crude oil</td>
<td>16</td>
<td>18.7</td>
<td>0.85</td>
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<tr>
<td>05</td>
<td>Jatropha biodiesel</td>
<td>15.7</td>
<td>18.7</td>
<td>0.83</td>
</tr>
<tr>
<td>06</td>
<td>Karanja crude oil</td>
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<tr>
<td>07</td>
<td>Karanja biodiesel</td>
<td>15.6</td>
<td>18.7</td>
<td>0.83</td>
</tr>
</tbody>
</table>

3.6. Transesterification

By trasesterification algal oil was converted to biodiesel, which is shown in Fig. 12. The upper layer shows biodiesel, and lower layer shows glycerine.

![Figure 12. Upper layer shows biodiesel and lower layer shows glycerin.](image)

4. Conclusions

The microalgal biofuel is technically feasible, and only renewable biofuel that can potentially replace liquid fuels derived from gasoline. Production of low-cost microalgal biofuels primarily requires improvements to microalgal biology through genetic engineering. Use of MBM medium was
better than CHU-13 at the laboratory scale. Micro-screening was the best method for algae harvesting in comparison to centrifugation and other method like floculation, and sieves size was 250 - 500 μm. For the extraction of oil, the best method was Soxhlet method in terms of reduce labor work (125 mL oil/500 g algae). In terms of save time, the best method was expeller method (115 mL/500 g). In terms of purity, the best method was ultrasonicator (108 mL/500 g). The cost effective and quantitative method was Soxhlet method, and it was the most reliable method for oil extraction. The extracted oil was further used for biodiesel production by transesterification process. The fatty acid and triglyceride were identified using thin layer chromatography and paper chromatography, and the RF values were calculated.

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References


