Scenedesmus as a potential source of biodiesel among selected microalgae

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Energy security has become a national issue and several attempts are being made to seek viable alternatives in the form of renewable energy to meet the future needs. Biofuel production from microalgae is considered as an effective strategy in this endeavour. Seven microalgae, viz. Chlorella, Haematococcus, Ulothrix, Chlorococcum, Scenedesmus, Rivularia and Scytonema were selected from 16 isolated cultures from six freshwater bodies. Ulothrix sp. reached a high growth rate of 0.42 ± 0.01 g/l and low lipid content of 5.56 ± 0.81% on the 15th day of incubation. Under similar conditions, Scenedesmus sp. reached a growth rate of 0.38 ± 0.01 and recorded a lipid content of 27.4 ± 0.75%. Algal oil samples were analysed by thin layer chromatography and Fourier transform infrared spectroscopy. The fatty acid composition was detected by gas chromatography. Scenedesmus sp. showed the highest amount of oleic acid (11.77 mg g⁻¹ dry wt). The results suggest that Scenedesmus sp. is useful for producing biodiesel, based on its high lipid and oleic acid contents.

Keywords: Biodiesel, fatty acid, growth analysis, lipid content, microalgae.

GLOBAL warming and the exhaustion of fossil fuels are major worldwide problems. Thus, the production of biodiesel using various materials, such as plants, microalgae and animal fat, has been attempted as an alternative energy source. Bioenergy is one of the most important components to mitigate greenhouse gas (GHG) emissions and for substitution of fossil fuels. The study of algae for fuel has become a hot topic in recent years with energy prices fluctuating widely and GHG emissions increasingly becoming a cause for concern.

Biomass has been focused upon as an alternative energy source, since it is a renewable resource and fixes CO₂ in the atmosphere through photosynthesis. If biomass is grown in a sustained way, its combustion has no impact on the CO₂ balance in the atmosphere, because the CO₂ emitted by the burning biomass is offset by the CO₂ fixed by photosynthesis. Among biomass, algae usually have a higher photosynthetic efficiency. Many algal species have been found to grow rapidly and produce substantial amounts of tricacylglycerol or oil, and are thus referred to as oleaginous algae. It has long been postulated that algae could be employed as cell factories to produce oils and other lipids for biofuels and other biomaterials. An accurate method for lipid quantification in algal biomass is necessary for the purpose of selecting optimum species and growth conditions. In this study, the algal growth rate, biomass production, lipid content and productivity of microalgal cultures isolated from freshwater bodies were determined. Furthermore, the fatty acid composition was detected using gas chromatography (GC), and algal oil samples analysed using thin layer chromatography (TLC) and Fourier transform infrared (FTIR) spectroscopy.

Water samples for microalgae isolation were collected from different sites (in and around Gandhigram Rural Institute-Deemed University, Gandhigram, Tamil Nadu, India) that appeared to contain algal growth in freshwater bodies. All samples were collected at about the same time between 0800 to 1100 h. Surface water and water at a depth of 0.50 m were collected at each location. Water
samples were taken from the sites to the laboratory in bottles cooled in ice. Next 10 ml of water samples were transferred to a 500 ml conical flask containing 200 ml of sterilized Bold’s Basal Medium (BBM)4 and incubated on a rotary shaker at 27°C and 100 rpm under continuous illumination using white fluorescent light (maximum 2500 lux) for three weeks. BBM was composed of (mg/l) NaNO3, 250; K2HPO4, 75; KH2PO4, 175; CaCl2, 25; NaCl, 25; MgSO4, 75; FeCl3, 0.3; MnSO4·7H2O, 0.3; ZnSO4·7H2O, 0.2; H3BO3, 0.2 and CuSO4·5H2O, 0.06. Every two days, the flasks were examined for algal growth using an optical microscope, with serial dilutions being made in BBM from the flasks showing growth. Subcultures were made by inoculation of 50 µl culture solution onto petri plates containing BBM solidified with 1.5% (w/v) of bacteriological agar. These procedures were repeated for each of the original flasks. Petri plates were incubated at 27°C under continuous illumination for two weeks. The purity of the cultures was confirmed by repeated plating and by regular observation under a microscope. The freshwater microalgae were identified and authenticated based on the guidelines of the standard manual5.

At first, cells of the identified microalgae were cultivated in 2 l flask using BBM and incubated batch-wise at 24°C for 18 days. The cultures were bubbled with sterile air and illuminated (2500 lux maximum). Every three days the microalgal cells were harvested by centrifugation and washed twice with deionized water. Microalgal pellets were dried at 80°C for dry weight measurements6. Experiments were performed in triplicate, and data expressed as mean ± standard deviation (±SD).

The total lipids were extracted from microalgal biomass using a modified method of Bligh and Dyer7. The lipids were extracted with chloroform–methanol (2 : 1, v/v), and then separated into chloroform and aqueous methanol layers by the addition of methanol and water to give a final solvent ratio of chloroform : methanol : water as 1 : 1 : 0.9. The chloroform layer was washed with 20 ml of a 5% NaCl solution, and evaporated using a rotary vacuum evaporator (Rotavapor R-210, Buchi). Experiments were performed in triplicate, and data expressed as mean ± standard deviation (±SD).

TLC screening for lipids was carried out by spotting a concentrated methanolic solution of the extract on silica gel plates. TLC plates were developed in ethyl acetate : methanol : water (40 : 5 : 4.4) mobile phase and air-dried. The iodine vapour was used to visualize the separated lipid compounds. The presence of lipid compounds was detected by brown spots against a white background, which are compared with the standard Jatropha oil8. This technique is used to confirm the oil which is extracted from microalgae.

FTIR spectroscopy was used to study the structure and chemical bonding of the algal oil, especially to identify the functional groups. FTIR attenuated total reflectance spectra were collected on a PerkinElmer Spectrum 400 FTIR instrument using a diamond smart iTR reflectance cell with a DTGS detector. Algal lipid did not require any preparation and were pressed against the diamond cell prior to scanning. Scenedesmus, Chlorella and Jatropha oil was taken for each spectrum based on potential of oil extraction. The spectra were collected in the range 4000–500 cm–1 (at 4 cm–1 resolution) and data were exported using Orgin 8.0 Microcal™ Inc. software.

A fatty acid profile analysis was performed using a Shimadzu 2010 gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a flame ionization detector and a diethylene glycol succinate capillary column (30 m × 0.25 × 0.25 µm). Both initial column temperature and injection port temperature were 180°C. Detector temperature was 230°C, and this was increased to 300°C at a temperature gradient of 15°C/min. Lipid samples (100 µl) was placed into capped test tubes, saponified with 1 ml of saturated KOH–CH3OH solution at 75°C for 10 min, and then subjected to methanolysis8 with 5% HCl in methanol at 75°C for another 10 min. Thereafter, the phase containing the fatty acid methyl esters was separated by adding 2 ml of distilled water, and methanol was recovered. The components were identified by comparing their retention times and fragmentation patterns with those of the standards9. Six fatty acid methyl esters (C16 : 1, C17 : 0, C18 : 0, C18 : 1, C18 : 2 and C18 : 3) were used as the standard materials.

Many algal species exhibit rapid growth and high productivity, and several microalgal species can be induced to accumulate substantial quantities of lipid, often greater than 60% of their dry biomass10. In this study, 16 microalgal cultures were isolated from six different water bodies. Only seven isolates (Chlorella, Haematococcus, Ulothrix, Chlorococcum, Scenedesmus, Rivularia and Scytonema) were selected based on their purity and growth rate (Table 1). Several thousand algae and cyanobacterial species have been screened for high lipid content, of which several hundred oleaginous species have been isolated and characterized under laboratory and/or outdoor culture conditions12.

Under suitable conditions and sufficient nutrients, microalgae can grow profusely13. Figure 1 shows the growth rate among the examined microalgal species.

**Table 1. Isolation of microalgae from different locations in and around Gandhimag**

<table>
<thead>
<tr>
<th>Location</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Microalga</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kamarajar dam</td>
<td>10°17’43.44″N</td>
<td>77°48’44.06″E</td>
<td>Chlorella sp.</td>
</tr>
<tr>
<td>Palar dam</td>
<td>10°24’30.61″N</td>
<td>77°29’38.39″E</td>
<td>Haematococcus sp.</td>
</tr>
<tr>
<td>Palani pond</td>
<td>10°26’12.59″N</td>
<td>77°30’52.27″E</td>
<td>Ulothrix sp.</td>
</tr>
<tr>
<td>Manjalar dam</td>
<td>10°11’37.15″N</td>
<td>77°37’55.86″E</td>
<td>Scenedesmus sp.</td>
</tr>
<tr>
<td>Nanganji dam</td>
<td>10°35’35.34″N</td>
<td>77°29’38.39″E</td>
<td>Rivularia sp.</td>
</tr>
<tr>
<td>Anaiappatti dam</td>
<td>10°05’20.15″N</td>
<td>77°51’10.28″E</td>
<td>Scytonema sp.</td>
</tr>
</tbody>
</table>
indicating the enhanced growth rate corresponding with the incubation time. The growth rate of the thalli reached its peak in 15 days of incubation. The biomass productivity as expressed as dry cell weight per litre indicated that the *Ulothrix* sp. (0.42 ± 0.01 g l⁻¹ dry wt) showed two times greater growth rate than *Chlorococcum* sp. (0.24 ± 0.02 g l⁻¹ dry wt).

After mass multiplication, the microalgae were harvested and used for oil extraction. Among the isolated microalgae, *Scenedesmus* sp. and *Chlorella* sp. produced high lipid content of 27.4 ± 0.75% and 22.3 ± 0.6% dry wt respectively (Figure 2). *Ulothrix* recorded low lipid content (5.56 ± 0.81%), although it produced high biomass (0.42 ± 0.01 g l⁻¹ dry wt) on the 15th day of growth. Liu *et al.*¹⁴ reported that total lipid content representing 20–50% of the dry biomass weight was found to be quite common, and some microalgae even exceeded 80%. The oil content of *Chlorella* typically ranges between 28% and 32% dry wt¹³, but can reach 46% dry wt under stress¹⁵. Chisti¹³ reported that oil content of microalgae is usually between 20% and 80%.

Four isolated microalgae (*Scenedesmus* sp., *Chlorella* sp., *Rivularia* sp. and *Haematococcus* sp.) were selected based on the potential of lipid compound. TLC screening method indicated the presence of lipid compounds. In this experiment the active compounds appeared as brown spots against a white background. The ATR–FTIR spectra of the pure lipid compounds of *Chlorella*, *Scenedesmus* and *Jatropha* are shown in Figure 3. The FTIR transmittance spectrum reveals the presence of hydroxyl, alkane, alkene and carbonyl groups in *Chlorella*, *Scenedesmus* and *Jatropha*. The bands at 3420 and 3436 cm⁻¹ due to the O–H stretching vibrations have been observed in the *Chlorella* sp. and *Scenedesmus* sp. respectively. The weak band centred on 2920 cm⁻¹ is due to the presence of asymmetric C–H stretching vibration. The observed bands around 2847 and 2681 cm⁻¹ correspond to the symmetric C–H stretching vibration in the *Chlorella* sp.
sp. and Scenedesmus sp. respectively. Three distinct bands were observed in the region 1641, 1310 and 1000 cm⁻¹, which reveals the presence of esters in the as prepared samples. The band corresponding to the C=O stretching vibration was observed at 1641 cm⁻¹ in the Chlorella sp. and Scenedesmus sp. The weak band observed 1360 cm –¹ is due to the presence of the C–C–O stretch in the present studied samples. The band centred at 1000 cm⁻¹ is mainly due to the availability of O–C–C stretching in both Chlorella sp. and Scenedesmus sp. Also, the bands corresponding to the asymmetric CO₂ stretch and symmetric CO₂ stretch were indentified between 1650 and 1540 cm⁻¹, and 1450 and 1360 cm⁻¹ respectively. The band observed at 832 cm⁻¹ is attributed to the bending of =C–H vibration. From these individual lipid spectra, it is clear that characteristic and distinct fingerprints for triglycerides and phospholipids exist in the FTIR spectrum of Scenedesmus sp. and Chlorella sp. This result is in agreement with several others¹⁶,¹⁷.

Fatty acids in the two microalgal strains were primarily esterified based on the potential of lipid content, and six fatty acid methyl ester profiles were determined using GC analysis (Table 2). In a previous study¹⁸, the most commonly synthesized fatty acids have chain lengths that range from C16 to C18, similar to those of higher plants, and palmitic, stearic, oleic and linolenic acids were recognized as the most common fatty acids contained in biodiesel. In the two tested microalgae, oleic acid (C18 : 1) and linoleic acid (C18 : 2) were dominant, which ranged from 19.9% to 52.8% and 43.2% to 74.4% respectively. The total amount of fatty acid methyl ester of the two microalgae ranged from 19.62 to 22.29 mg g⁻¹ dry wt. The highest amount of oleic acid (11.77 mg g⁻¹ dry wt) was detected in Scenedesmus sp., while linoleic acid (14.61 mg g⁻¹ dry wt) was higher in Chlorella sp. Oils with high oleic acid content have been reported to have a reasonable balance of fuel, including their ignition quality, combustion heat, cold filter plugging point, oxidative stability, viscosity and lubricity, which are determined by the structure of their component fatty esters¹⁹. Thus, it was concluded that among the tested microalgal species, Scenedesmus sp. showed the highest oleic acid content (52.8%), making it most suitable for the production of good quality biodiesel.

This study showed certain freshwater microalgae having high growth rate and lipid productivity; seven microalgal cultures were shortlisted based on purity and growth rate. The highest growth rate (0.42 ± 0.01 g l⁻¹ dry wt) was found for Ulothrix sp. on the 15th day of incubation. The presence of lipid compounds among the examined algae was substantiated by the findings of TLC and FTIR. The highest total fatty acid and lipid contents of 22.29 mg g⁻¹ dry wt and 27.4 ± 0.75% respectively, and oleic acid (11.77 mg g⁻¹ dry wt) was mainly found in Scenedesmus sp. The results of this study indicate that the naturally isolated microalga Scenedesmus sp. is valuable for use in oil production.

Table 2. Fatty acid composition of Scenedesmus sp. and Chlorella sp.

<table>
<thead>
<tr>
<th>Fatty acid methyl ester</th>
<th>Amount of fatty acids (mg g⁻¹ dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scenedesmus sp.</td>
</tr>
<tr>
<td>C16 : 1</td>
<td>ND</td>
</tr>
<tr>
<td>C17 : 0</td>
<td>0.21 (0.94)</td>
</tr>
<tr>
<td>C18 : 0</td>
<td>0.59 (2.64)</td>
</tr>
<tr>
<td>C18 : 1</td>
<td>11.77 (52.8)</td>
</tr>
<tr>
<td>C18 : 2</td>
<td>9.63 (43.2)</td>
</tr>
<tr>
<td>C18 : 3</td>
<td>0.09 (0.4)</td>
</tr>
<tr>
<td>Total</td>
<td>22.29 (100)</td>
</tr>
</tbody>
</table>

ND, Not detected. Numbers with brackets represent fatty acid methyl ester composition (wt%).


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