FULL LENGTH ARTICLE

Evaluation of the potential for some isolated microalgae to produce biodiesel

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Abstract The energy and the world food crises have ignited interest in algal culture for making biodiesel, bioethanol, biobutanol and other biofuels using the land that is not suitable for agriculture. Algal fuel is an alternative to fossil fuel that uses algae as its source of natural deposits. Microalgal lipids are the oils of the future for sustainable biodiesel production. One of the most important roles in obtaining oil from microalgae is the choice of species. A total of fifteen microalgal isolates, obtained from brackish and fresh waters, were assayed at the laboratory for their ability to high biomass productivity and lipid content. Only three microalgae were selected as the most potent isolates for biomass and lipid production. They have been identified as Chlorella vulgaris, Scenedesmus quadri and Trachelomonas oblonga. All of them were cultivated on BG11 media and harvested by centrifugation. The dry weight of the three isolates was recorded as 1.23, 1.09 and 0.9 g/l while the lipid contents were 37%, 34% and 29%, respectively which can be considered a promising biomass production and lipid content.

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1. Introduction

Depletion of world petroleum reserves and the impact of environmental pollution by increasing exhaust emissions have led to the search for suitable alternative fuels for diesel engines [1]. Biodiesel is an alternative to diesel fuel, which is produced from oils via transesterification. Currently, it is being recognized as a green and alternative renewable diesel fuel that has attracted vast interest from researchers, governments, and local and international traders [2]. It is nontoxic, biodegradable and has the potential to replace the conventional diesel fuel. Presently, biodiesel is produced from different crops, such as, soybean, rapeseed, sunflower, palm, coconut, jatropha, karanja, used fried oil and animal fats [3]. There will be certain limitations in the use of these oils as alternate fuels because of its food demand, life span, lower yield, higher land usage and higher price inter alia [4]. It is necessary to search for non food based alternate feedstocks for biodiesel production. Selection of biodiesel feedstock is based on higher yields, short duration, lower production cost and less land usage. Among various biodiesel feedstocks, the microalgae oil has the

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potential to replace the conventional diesel fuel. Microalgae have been suggested as a potential feedstock for fuel production because of a number of advantages, including higher photosynthetic efficiency, higher biomass production, and higher growth rates, as compared to other energy crops [5]. The interest in microalgae for oil production is due to the high lipid content of many species, and also to the fact that lipid synthesis, especially of the non-polar triacylglycerols (TAGs), which are the best substrate to produce biodiesel, can be modulated by varying growth conditions. Biodiesel production from microalgal biomass is a sequential process that consists of the cultivation, harvest, oil extraction, and conversion of algal lipids into advanced biofuels [4]. A key consideration is the choice of algal strain. The growth characteristics and composition of microalgae are known to significantly depend on the cultivation conditions. There are four major types of cultivation conditions for microalgae: photoautotrophic, heterotrophic, mixotrophic and photoheterotrophic cultivation [6]. Phototrophic cultivation occurs when the microalgae use light, such as sunlight, as the energy source, and inorganic carbon (e.g., carbon dioxide) as the carbon source to form chemical energy through photosynthesis [5]. This is the most commonly used cultivation condition for microalgae growth [7,8]. Harvesting of microalgae is seen as one of the major challenges of using microalgae for the production of biodiesel. Microalgae that store lipids have low densities and are found in suspension making separation difficult. Large scale extraction procedures for microalgal lipids are complex and still in the developmental stage [9]. Microalgal oil can be extracted chemically or mechanically, similar to other oleaginous biomass. Usually physical extraction requires an additional chemical as a solvent to enhance the extraction process. The most popular solvents include hexane or chloroform and alcohol. The combination of polar and non-polar solvents enhances the extraction of both polar and non-polar lipids. Crude microalgal oil is especially high in viscosity, thus requiring conversion to lower molecular weight constituents in the form of fatty acid alkyl esters. Transesterification converts raw microalgal lipid (triacylglycerols/free fatty acids) into renewable, non-toxic and biodegradable biodiesel for direct consumption by unmodified diesel engines [9]. This research is an attempt to grow and develop different microalgal strains having promising dry weight and lipid content to produce valuable biodiesel.

2. Materials and methods

2.1. Samples: Collection and analysis

Water samples used to isolate microalgae were collected aseptically from sites that appeared to contain algal bloom. About eight different water samples were collected from different locations in Egypt, four of them from Giza Governorate at different sites in Mariota symbolized as (Pm, Spm, Dpm and Cm), one from Sharqawia canal in Qaliubia Governorate (Scq) and one from Canal water in Qaliubia (Ck). Another sample was collected from Cairo Governorate Kupri El kuba (Gk) and also one from Ain Elsira (As). Samples were gathered from about a diameter of 10 cm under the water surface and placed in sterile plastic bags, then transported to the laboratory within 24 h of collection.

2.2. Physical and chemical analyses of water samples

The Physical and chemical properties of the water samples were determined at Central Lab, Egyptian Petroleum Research Institute. Anions and cations were determined according to ASTM D-4327 and 6919, respectively using an ion chromatography. The instrument used was Dionex IC model 1100 equipped with high capacity columns (AS9 and CS12) for anions and cations respectively, TDS was determined according to ASTM D-1893 using a digital pH meter model metler Toledo-Seven Go. Alkaline species (CO3\text{2-}, OH, HCO3\text{-}) were measured according to ASTM D-3875. Calculations were done using Alkalinity calculator Ver. 2.10 (USGS).

2.3. Isolation, purification and identification of microalgae

Ten ml of water sample was transferred to a 500 ml conical flask containing 250 ml of sterilized BG 11 medium [10]. The flasks were incubated on a rotary orbital shaker at 150 rpm under continuous illumination using white fluorescent light at intensities of 3000 Lux for three weeks. Every two days, the flasks were examined for algal growth using an optical microscope. Subcultures were made by inoculating 50 µl of culture solution onto Petri plates containing the same isolation media solidified with 1.5% (w/v) of bacteriological agar. The purity of the culture was confirmed by repeated plating, also by repeated observation under a microscope. The obtained isolates were identified microscopically according to Prescott [11].

2.4. Determination of microalgal growth

After the Microalgal cultivation on BG11 medium under the previous conditions, the microalgae growth was determined by measuring optical density at a wavelength of 685 nm [12] (denoted as OD 685) using a spectrophotometer (model Jenway 6300, Eu). The dry cell weight (DCW) of microalgae biomass was also obtained by filtering 50 ml of aliquots of culture through a cellulose acetate membrane filter (0.45 µm pore size, 47 mm in diameter). Each loaded filter was dried at 105°C until the stability of weight is reached. The dry weight of the blank filter was subtracted from that of the loaded filter to obtain the microalgae dry cell weight.

2.5. Lipid extraction and fatty acid analyses

The total lipids were extracted from the fresh microalgal biomass using a slightly modified method of Bligh and Dyer [13]. In brief, 50 ml of microalgal culture was harvested by centrifugation at 10,000 rpm for 5 min, re-suspended in 1 ml of distilled water. After drying the samples using oven, the samples were extracted using a mixture of chloroform: methanol (1:1. v/v). The mixtures were transferred into a separating funnel and shaken for 5 min, an additional portion of chloroform (the same volume) was added and the extraction mixture was shaken again for 5 min. To separate the chloroform and aqueous methanol layers, a same volume of water was added and then centrifuged at 10,000 rpm for 10 min. The chloroform layer was gently removed from the bottom, and a second extraction was performed. The chloroform portions were
collected and washed with 5 ml of 5% NaCl solution and evaporated in an oven at 50 °C to dryness. Thereafter, the weight of the crude lipid obtained from each sample was measured gravimetrically.

2.5.1. Fatty acid analysis

The fatty acid profile of the extracted oil sample of all species was determined by converting the fatty acids in the oil to fatty acid methyl esters (FAMEs). The FAME composition was determined using a Gas-Chromatography (GC) with a split automatic injector and silica capillary column DB-5 (length: 60 m; ID: 0.32 mm.). Details of the procedure have been described according to Lepage and Roy (1986 and 1988) [14,15]. Helium was used as carrier gas at a flow rate of 1 ml/min. The column was held at 150 °C for 1 min and ramped to 240 °C at a rate of 30 °C/min, and it was then held at 240 °C for 30 min. Standards were used to give rise to well individualized peaks that allow the identification of the fatty acid composition.

3. Results and discussion

3.1. Samples, collection and analysis

Microalgae are ubiquitous organisms present in all existing earth ecosystems, not only aquatic, but also terrestrial, representing a large variety of species living in a wide range of environmental conditions [16]. The physical and chemical properties results of eight water samples from different sites were determined at Central Lab, Egyptian Petroleum Research Institute which are shown in Table 1.

The concentration of dissolved solids (TDS) in stream water is important because it determines the flow of water in and out of the cells of aquatic organisms. Aforesaid output data showed the variation of TDS results which revealed the difference of water sample nature, for example, the high TDS value for AS, GK, PM, SPM and DPM samples means that it is brackish water, while its value for SCQ, CM and TDS value for AS, GK, PM, SPM and DPM samples means because of its behavior stability.

In our study, more than twenty-eight isolates were isolated from the collected water samples but only fifteen axenic microalgae isolates were selected and sub-cultured on slants on its specific isolation media (BG11) and kept in a refrigerator for further investigation due to their purity. According to morphological examination under a microscope based on cell shapes, fifteen microalgal isolates were identified as, *Chlorella vulgaris* Pm, *Scenedesmus quadricauda* Scq, *Microcystis aeruginosa* Spm, *Chlorella sp.*Spm3, *Chlorella sp.*Spm5, *M. aeruginosa* Dpm *Chlorella sp.* Cm, *Chlorella sp.* Ck, *Trachelomonas oblonga* Ck, *M. aeruginosa* Ck, *Haematococcus pluvialis* Gk, *M. aeruginosa* As, *Chlorella sp.* Scq, *M. aeruginosa* Gk, *Chlorella sp.* Dpm, respectively.

N.B: The symbol after algal name refereed to the isolation place.

3.3. Biomass and lipid content

One of the most important decisions in obtaining oil from microalgae is the choice of species. Accordingly; all the fifteen purified strains were screened for their lipid content and mass productivity (Figs. 1 and 2). Among all isolates, *C. vulgaris* Pm, *S. quadricauda* Scq and *T. oblonga* Ck were the most potent isolates. The dry weight of the three isolates were recorded as 1.23, 1.09 and 0.9 g/l while the lipid contents were 37%, 34% and 29%, respectively which can be considered a promising biomass production and lipid content. Rodolfi et al. (2009) and Reda et al. (2011) recorded that lipid content of fresh water microalgae was nearly 20% [19,20]. Several microalgae species can be induced to accumulate substantial lipid quantities to obtain high oil yields. However, some differences exist among various species, and even within the same genus [21]. From Figs. 1 and 2, it can be clearly observed that, some isolates may be similar to *T. oblonga* or slightly more in dry weight and lipid content but this strain was selected because of its behavior stability.

3.4. Growth rate of microalgal strains

Under suitable conditions and sufficient nutrients, microalgae can richly grow. Usually, they double their biomass within

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Physical and chemical properties of the collected water samples.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water sample</td>
<td>AS</td>
</tr>
<tr>
<td><strong>Analysis</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Physical properties</strong></td>
<td></td>
</tr>
<tr>
<td>Total dissolved solids (TDS) mg/l</td>
<td>8493</td>
</tr>
<tr>
<td>pH @ 25°C</td>
<td>8.3</td>
</tr>
<tr>
<td>Salinity mg/l</td>
<td>3839.6</td>
</tr>
<tr>
<td>Hardness mg/l</td>
<td>2822.1</td>
</tr>
<tr>
<td><strong>Chemical properties (mg/l)</strong></td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.34</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.02</td>
</tr>
<tr>
<td>Chloride</td>
<td>2327</td>
</tr>
<tr>
<td>Sodium</td>
<td>1472</td>
</tr>
<tr>
<td>Magnesium</td>
<td>217.8</td>
</tr>
</tbody>
</table>
3.5 h or 24 h during the exponential growth phase [22]. The pure growth rate differed among the examined microalgal species (Fig. 3). Algal growth is directly affected by the availability of nutrients, light, the stability of pH, and temperature [23].

Under similar environmental conditions, the average specific growth rates of 5.728 and 5.525 were found for S. quadricauda Scq and T. oblonga Ck respectively at 680 nm after 29 day incubation compared with 11.721 for C. vulgaris Pm. This result indicates that, C. vulgaris Pm, S. quadricauda Scq and T. oblonga Ck strains were suitable for high-density cultures.

3.5. Fatty acid composition

Fatty acid compositions determine biodiesel properties, owing to the chemical features of fatty acids, such as carbon chain length and unsaturation extent. Therefore, fatty acid profiles for the most potent strains were determined (Table 2). The most important unsaturated fatty acids present in microalgal

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Chlorella vulgaris Pm</th>
<th>Scenedesmus quadricauda Scq</th>
<th>Trachelomonas oblonga Ck</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>1.95</td>
<td>3.9</td>
<td>2.78</td>
</tr>
<tr>
<td>C14:0</td>
<td>1.88</td>
<td>1.76</td>
<td>1.49</td>
</tr>
<tr>
<td>C14:1</td>
<td>ND</td>
<td>ND</td>
<td>2.48</td>
</tr>
<tr>
<td>C16:0</td>
<td>10.35</td>
<td>22.02</td>
<td>15.86</td>
</tr>
<tr>
<td>C16:1</td>
<td>10.75</td>
<td>3.77</td>
<td>10.37</td>
</tr>
<tr>
<td>C16:2</td>
<td>7.27</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C17:0</td>
<td>12.94</td>
<td>2.11</td>
<td>12.35</td>
</tr>
<tr>
<td>C17:1</td>
<td>ND</td>
<td>1.8</td>
<td>1.03</td>
</tr>
<tr>
<td>C18:0</td>
<td>6.53</td>
<td>4.84</td>
<td>4.67</td>
</tr>
<tr>
<td>C18:1</td>
<td>6.80</td>
<td>25.97</td>
<td>8.42</td>
</tr>
<tr>
<td>C18:2</td>
<td>26.28</td>
<td>15.25</td>
<td>30.00</td>
</tr>
<tr>
<td>C18:3</td>
<td>10.45</td>
<td>12.05</td>
<td>8.02</td>
</tr>
<tr>
<td>C20:0</td>
<td>2.21</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Unsaturated</td>
<td>35.85</td>
<td>34.67</td>
<td>37.16</td>
</tr>
<tr>
<td>Total even</td>
<td>84.44</td>
<td>89.6</td>
<td>76.1</td>
</tr>
</tbody>
</table>

ND: Undetectable.

Figure 1  Dry weight of the fifteen microalgal isolates.

Figure 2  Lipid content of the fifteen microalgal isolates.

Figure 3  Growth curve of the three most potent microalgal strains.

Table 2  Fatty acid profile (% of total FAMEs) (Saturated and unsaturated fatty acids) of three most potent microalgal strains.
strains are, palmitoleic acid (C16:1), oleic acid (C18:1), lenoleic acid (C18:2) and linolenic (C18:3). These results comply with Knothe, 2008 who said that oleic acid, palmitoleic and palmitic acid were recognized as the most common fatty acids contained in microalgal lipid [24]. Oleic acid was found in the high concentration which reached to 25.97% for **S. quadricauda** Scq, and lenoleic acid was high in **T. oblonga** Ck reaching up to 30.00%. Palmitoleic acid, oleic acid, lenoleic acid and linolenic acid were found in all algal species. Oils with high oleic acid contents have been reported to have a reasonable balance of fuel, including its ignition quality, combustion heat, cold filter plugging point (CFPP), oxidative stability, viscosity, and lubricity, which are determined by the structure of its fatty esters component [25,24]. Therefore, among the tested microalgal species, **S. quadricauda** Scq showed the highest oleic acid content, making it the most suitable for the production of good quality biodiesel.

4. Conclusions

The total lipid content and net biomass productivity in microalgae vary greatly from one species to another although they belong to the same algal group. So, it is very important to screen microalgal strains before the selection of the suitable strain for the application. Three strains from 15 microalgal isolates were selected due to their high lipid content, mass production and ease of cultivation; they are **C. vulgaris** Pm, **S. quadricauda** Scq and **T. oblonga** Ck. The composition of fatty acids in the studied species was mainly C12:0, C16:0, C16:1, C18:1, C18:2 and C18:3. The results of this study indicate that the naturally isolated microalgal **C. vulgaris** Pm, **S. quadricauda** Scq and **T. oblonga** Ck are valuable candidates for use in biodiesel production.

References