Chlorella sp.: A new strain with highly saturated fatty acids for biodiesel production in bubble-column photobioreactor

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A B S T R A C T

The biodiesel production from a naturally isolated strain of Chlorella in 2 L bubble-column photobioreactor was studied. The microalgal strain was isolated from the rice paddy-field soil samples during a screening program. After 17 days, at the end of exponential phase of growth, the total content of the lipids was extracted. The extracted fatty acids were first esterified and then identified using GC/MS analysis. Several types of fatty acid methyl esters (FAMEs) were identified in the isolated microalga and the presence of saturated fatty acids in Chlorella sp. MCCS 040 was approved. The composition of fatty acids in the studied species of microalga was mainly palmitic acid methyl ester, myristic acid methyl ester, stearic acid methyl ester and undecanoic acid methyl ester. This strain because of its highly saturated fatty acids content can be an ideal candidate for biodiesel production.

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1. Introduction

Recently, fuel production from algae has been receiving considerable attention because of growing energy prices, emissions of greenhouse gases [1] and gradual depletion of fossil fuels [2]. Microalgae due to their rapid biomass production, high photosynthetic efficiency [1] and ability to storage a large amount of lipid are ideal source of biodiesel [3].

Averagely, microalgae can produce biodiesel 10–20 times higher than that obtained from oleaginous seeds and/or vegetable oils [4]. To assess the feasibility of producing biodiesel, a large amount of microalgae biomass is needed [5].

Microalgae can be normally cultivated in the open-culture systems (lakes or ponds) and also in the closed-culture systems called photobioreactors (PBRs) [6]. Biomass production of up to 150 tons ha−1 year−1 has already been reached in photobioreactor [7]. Experimental photobioreactors are often designed as columns [8]. Vertical bubble-columns have good light–dark cycling, low surface/volume and a much more chaotic gas–liquid flow [6].

Chlorella is widespread in different locations such as fresh water, air and soil [9]. Chlorella sp. has good potential to accumulate more than 20% lipids, such as C 18:1, C 16:0 and C 18:3 [10]. In this study a naturally isolated strain of Chlorella collected from soil samples, was cultivated in 2 L bubble-column photobioreactor and investigated for biodiesel production.

2. Materials and methods

2.1. Bioreactor design

The constructed photobioreactor has a total volume of 2 L (Fig. 1). The photobioreactor consisted of different parts. Glass-made cylinder, four plastic tubes with a diameter of 6 mm which were used for gas inlet, gas outlet, feeding and sampling, porous bulk stone which was used for bubbling the air, air pump, top head plastic cover with four ports for plastic tubes.

2.2. Growth and fatty acid composition of Chlorella

The inoculation culture of Chlorella was cultivated in the appropriate BG-11 liquid medium in 250 mL Erlenmeyer flasks containing 100 mL culture. Then Chlorella sp. was grown under low illumination (37 µmol m−2 s−1) and unlimited aerated condition for one week. Temperature was adjusted at 25 ± 2 °C.
2.2.1. Cultivation in photobioreactor

After one week, 50 mL of an inoculation culture was transferred to 2 L bubble-column photobioreactor containing 1 L of BG-11 liquid medium. Filtered air was continuously pumped through multi nozzles bulk stone at the bottom of photobioreactor. The aeration was performed at flow rate of 9 L min\(^{-1}\) for 24 h. The photobioreactor was illuminated with 40 W white fluorescent lamps which were placed at three side of the column and the average light intensity was approximately 37 l mol m\(^{-2}\) s\(^{-1}\). This run lasted for 17 days until Chlorella sp. cells reached the stationary phase. After finishing cultivation, culture medium was centrifuged at 5000 rpm, 4\(^\circ\)C for 5 min, and the cell pellets were freeze-dried. Then alga pellets were prepared for fatty acid extraction. Moreover, the dry weight of the algal cells was measured.

2.2.2. Growth parameters

The cell number and optical density was measured during the cultivation period in the photobioreactor. For cell count, each time 1 mL of algal suspension was removed through sampling tube and then direct count was performed using Neubauer hemocytometer and an Olympus Light Microscope. Also, optical density was measured at 670 nm using a UV/Visible spectrophotometer (PG instrument Ltd.). Growth curve is shown in Fig. 2.

2.2.3. 18S rRNA PCR amplification

18S rRNA gene sequence of Chlorella sp. MCCS 040 was amplified using two sets of primers [11]. The applied PCR condition has been described before [11]. The PCR products were electrophoresed in a 1% (w/v) agarose gel. The sequence was determined by the CinnaGen Company with the primers.

2.2.4. Multiple alignment analysis of the amplified sequence

We applied the NCBI databases with BLAST search to search sequence similarity (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.2.5. Fatty acid extraction, esterification and GC/MS analysis

Fatty acids extraction, esterification and GC/MS analysis protocol was described in our previous study [12].

3. Results

The biomass concentration of Chlorella sp. at the end of exponential phase was approximately 1.9 g/L. It has doubling time of approximately 15 h during the exponential phase. The BLAST results showed 99% similarity to the 18S small subunit rRNA of some Chlorella species. The profile of FAMEs was identified through the comparison of their mass spectra with those in Wiley libraries. The results are displayed in Table 1. Several types of FAMEs were detected through GC/MS analysis. These are as follows: methyl tetradecanoate, methyl decanoate, methyl dodecanoate, methyl undecanoate, methyl hexadecanoate, methyl heptadecanoate, methyl octadecanoate, methyl pentadecanoate (Table 1).

4. Discussion

The main goals of microalgae oil production are high lipid yield and high biomass productivity which can affect production costs [13]. The quality of biodiesel is dependent on composition of the fatty acid methyl esters [14]. Fatty acid methyl esters properties are determined by length of carbon chain, degree of unsaturation and the alcohol content of composed fatty acids [2]. Cetane number, cold-flow properties, oxidative stability and iodine value are the most important properties of biofuel which are derived from its fatty acid methyl esters structure [2]. Palmitic acid and stearic acid are known as the most common fatty acids contained in biodiesel [15]. Different types of saturated fatty acids were detected in the Chlorella sp. MCCS 040. Palmitic acid, stearic acid, myristic acid and undecanoic acid are the most fatty acid composition of the investigated strain. Also pentadecanoic acid, margaric acid, lauric acid and capric acid were determined in Chlorella sp. MCCS 040. Several types of fatty acids with different degree of unsaturation

Table 1

<table>
<thead>
<tr>
<th>FAMEs</th>
<th>Common name</th>
<th>Formula</th>
<th>FAME content (% total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl tetradecanoate</td>
<td>Methyl myristate</td>
<td>C₁₄H₂₈O₂</td>
<td>25.9</td>
</tr>
<tr>
<td>Methyl decanoate</td>
<td>Methyl caprate</td>
<td>C₁₀H₂₀O₂</td>
<td>1.1</td>
</tr>
<tr>
<td>Methyl dodecanoate</td>
<td>Methyl iuate</td>
<td>C₁₂H₂₄O₂</td>
<td>5.9</td>
</tr>
<tr>
<td>Methyl undecanoate</td>
<td>Methyl palmitate</td>
<td>C₁₄H₂₈O₂</td>
<td>17.7</td>
</tr>
<tr>
<td>Methyl hexadecanoate</td>
<td>Methyl margarate</td>
<td>C₁₆H₃₂O₂</td>
<td>23.6</td>
</tr>
<tr>
<td>Methyl heptadecanoate</td>
<td>Methyl stearate</td>
<td>C₁₃H₂₆O₂</td>
<td>2.1</td>
</tr>
<tr>
<td>Methyl octadecanoate</td>
<td>Methyl margarate</td>
<td>C₁₄H₂₈O₂</td>
<td>12.8</td>
</tr>
<tr>
<td>Methyl pentadecanoate</td>
<td>Methyl stearate</td>
<td>C₁₅H₃₀O₂</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic diagram of a bubble-column photobioreactor: (a) side view and (b) top view of the photobioreactor. F: feeding port, S: sampling port, In: gas inlet, Out: gas outlet.

Fig. 2. Growth profile in cell number and optical density of Chlorella sp.
in various microalgae have been reported [2,4,13–16]. Most of algal oils have high polyunsaturated fatty acids content with four or more double bonds [2]. As reported before 50–65% of microalgal (Chlorella vulgaris, Spirulina maxima, Nannochloropsis oleoabundans, Scenedesmus obliquus and Dunaliella tertiolecta) fatty acids were mainly composed of unsaturated fatty acids [17]. In compare with our previous study, palmitic acid content (23.6%) is more than four times higher than that of Chlamydomonas MCCS 029 (5.6%). Dodecanoic acid, tetradecanoic acid and pentadecanoic acid content of Chlorella sp. MCCS 040 was 5.9%, 25.9% and 6.3% respectively, which was higher than that of Chlamydomonas MCCS 029 (4.2%, 6.6% and 5.4%) [12]. Highly saturated fatty acids give an excellent cetane number and oxidative stability to biodiesel [17]. Biodiesel quality is directly related to cetane number which shows ignition quality in engine [18]. As the results show, only saturated fatty acids were detected in Chlorella sp. MCCS 040.

Palmitic acid, stearic acid and myristic acid content in the microalgal strain is higher than Chlorella protothecoides 0710 [19,20], Chlorella vulgaris (UTCC 90), Dunaliella tertiolecta (UTCC 420), Phormidium sp., and Scenedesmus obliquus (UTCC 5) [21]. Capric acid which its presence can improve biodiesel properties [14] has the content of 1.1% in the studied strain. Its content is higher than Chlorella vulgaris (UTCC 90), Dunaliella tertiolecta (UTCC 420) and Phormidium sp [21].

All of the oils extracted from algae are not suitable or compatible to use as biodiesel [2]. The most common vegetable oils that are used for biodiesel are C18 and C16 [2]. According to the results, Chlorella sp. MCCS 040 has some features that make this strain ideal for biodiesel production. These features are as follows:

1. All of its fatty acids are saturated which give good cetane number and oxidative stability to biodiesel.
2. Palmitic acid and stearic acid content which are known as the most common fatty acids contained in biodiesel, are present in this strain.
3. The hydrocarbon chain length of fatty acids is between C10 and C18.
4. This strain can be easily cultivated in photobioreactor with good biomass productivity.

5. Conclusion

The results of this study show that Chlorella sp. MCCS 040, according to its fatty acid content is an ideal candidate for biodiesel production. It is for the first time that such microalgae with highly saturated fatty acids and none monounsaturated and none polyunsaturated fatty acids has been reported. So that further investigation should be done on this strain to make it as a commercial biodiesel producer.

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References