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# Fatty Acids Composition of Microalga *Botryococcus* Sp. Cultured in Synthetic Medium

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**Abstract:** In response to the crisis of fossil fuel depletion and global climate change, microalgae cultivation has received significant attention as an alternative to sustainable biodiesel production. In this study, the total fatty acids content was quantified from *Botryococcus* sp biomass grown in synthetic medium Bold Basal Medium (BBM) under laboratory conditions. The microalgal biomass was harvested through centrifugation in the late logarithmic growth phase and then it was freeze-dried at -40$^{o}$C and 0.12 Mbar for 86400 s (24 hrs). The lipids were extracted following Soxhlet method, and the fatty acids were analyzed using GC–MS. From the results obtained, the fatty acid composition is; C16:0 (Palmitic acid) contributing 27.950%, followed by C18:3 (stearic acid 22.758%), followed by C 18:2 (linoleic acid17.046) and the lowest is C 15:1 (pentadecanoic acid) with 0.051%. Most of the fatty acids obtained are both saturated and unsaturated which are similar to the conventional biodiesel and diesel properties making this green microalga *Botryococcus* sp. a desirable feedstock for biodiesel production. Thus, this locally isolated *Botryococcus* sp. has a high potential to be used as a source of biodiesel in the future.

**Keywords:** Biodiesel; *Botryococcus* sp.; Fatty acids; TNJER and Microalgae.

## 1. Introduction.

For decades, fossil fuel has played a vital role in the fast growth of economies in countries worldwide. Due to high demand, there is a rapid reduction of fossil fuel leading to an instability of the prices and has left a negative impact on the ecosystem. These issues have prompted a search for alternative sources of energy [1]. Microalgae have a significantly higher photosynthetic efficiency 10–20 times higher than terrestrial plants by generating much biomass within a short period [2, 3]. Microalgae grow well in several environments including aqueous suspensions therefore they do not need arable land like food crops. They can also sequester carbon dioxide from the atmosphere [4, 5]. Also, microalgal biomass is helpful in wastewater treatment [6] and the production of a broad range of fuels such as hydrogen, methane, syngas, liquid fuels and some biochemicals [7]. However, there has been a growing interest in microalgae as a promising candidate for the renewable energy source in the production of biofuel [8]. Microalgae can produce as much as 20 - 80% of lipids from its dry cell weight which makes it an excellent alternative source for the production of biodiesel, despite these appealing advantages, microalgae-based fuel production remains outside the realm of economic feasibility [9, 10]. [11] reported the cultivation of the green microalga, *Botryococcus* sp. under normal conditions to yield a small biomass albeit a high lipid content, making it an unsuitable source for large-scale biodiesel production. However, when microalgae are cultivated under certain conditions, they can yield more lipid content. Especially when grown under a low nitrogen (N) and phosphorus (P) or other stressed conditions the growth rate will be small, but their production of lipid will be higher [12]. Several growth media for this alga has been reported and they include; modified Chu 13, Blue Green (BG-11) and Bold basal medium (BBM) [13].

Lipid from any species of microalgae has a fatty acid profile that corresponds to vegetable oil; therefore, it can be tapped for the

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production of biodiesel [14]. Lipid content can be variable for the same algal strain, therefore, in selecting a microalga specie for biodiesel production, it is essential to look at both the lipid it produces and its fatty acid profile. An optimal proportion of saturated to unsaturated fatty acids in the fatty acid methyl ester (FAME) yields the best quality of biodiesel [15]. Therefore, the primary goal of the present study is to determine the fatty acids composition of the oil portion of this *Botryococcus* sp. as a potential sustainable feed-stock in large-scale production of biodiesel.

## 2. Materials and Method

### 2.1 *Botryococcus* sp. isolation and identification

The freshwater microalga, *Botryococcus* sp. used in this study was isolated from *TNJER* (Taman Negara Johor Endau-Rompin) located in the southern region of Peninsular Malaysia (2.4203° N, 103.2614°). The preparation of *Botryococcus* sp. growth medium follows [16]. Cell observation and concentration count was carried out using Neubauer hemacytometer chamber while molecular identification follows [17].

### 2.2 Microalga cultivation

The isolated microalga was cultured under a synthetic medium at research building of University of Nottingham Malaysia Campus. Growth experiments were conducted at ambient temperature in 500 mL- Erlenmeyer flasks. Stock solutions were prepared following Bold's Basal Medium (BBM). All the nutrients used in this study are of analytical grade supplied by Fisher Scientific Sdn. Bhd. (Selangor, Malaysia). Media was prepared by adding 2.0 mL of the stock solution and 0.2 mL into 100 mL of deionized water and subsequently refilled to 200 mL by addition of deionized water. The media and flasks were sterilized in an autoclave at 121$^{o}$C for 900 s. Inoculation of microalgae seed based on cell density $10^3$, $10^4$ and $10^5$ cells/mL were carried out by taking samples from stock culture ($9x10^6$ cells/mL). Balls of sterile cotton were plugged into the flasks and they were kept under room conditions for cultivation. Each autotrophic batch culture was carried out in triplicates for 14 days, and the system was shaken periodically for homogenization [16]. The specific growth rate of the culture was measured using equation 1 where Xf and Xi are the final and initial cell concentrations (cells/mL), Tf and Ti final and initial times respectively.

$$\text{Specific growth rate (Day}^{-1}) = \frac{Ln\frac{Xf}{Xi}}{Tf-Ti} \quad (1)$$

### 2.3 Extraction and analysis of fatty acids

The microalgal biomass was harvested by phase separation in a centrifuge (Eppendorf™ 5430, Fisher Scientific Sdn. Bhd. Selangor, Malaysia) at a speed of 6500 rpm for 15 min. The resulting solid pellets of microalgae sample was further washed with deionized water several times and subsequently freeze-dried in a freeze dryer (Martin Christ Alpha LD2, Germany) at -40$^{o}$C and 0.12 Mbar for 86400 s (24 hrs). Then it was weighed to get the dry biomass weight. Lipid extraction was done from 500 mg of dried biomass with hexane using soxhlet extraction method. The extraction sample preparation follows [17]. The extracted lipid was stored for lipid profiling through fatty acid analysis. Briefly, the fatty acids through the process of direct transmethylation of the extracted lipid were converted into methyl esters. The fatty acid methyl esters obtained were then analyzed by Perkin Elmer Clarus 680SQ8 Gas Chromatography system (USA) equipped with a flame ionization detector (FID).

## 3. Results and Discussion

### 3.1 Growth of *Botryococcus* sp.

The BLAST database found a high similarity to *Botryococcus* sp. at about 92%, with an accession number of JQ585723.1.The growth of *Botryococcus* sp. was measured during the period of cultivation by a hemocytometer and was expressed as cell density (cells/mL) [18]. The *Botryococcus* sp. maximum growth was found to be highest at the seventh day with 288 $x10^4$ cells/mL, and the specific growth rate is 0.81 day$^{-1}$. Fig. 1 also clearly shows that there is gradual increment from day 1 of cultivation up to day 7. The growth decreased from day 10 till day 14, to 5.6 x $10^5$ cells/mL. These findings are similar to the results of earlier studies by [16,18,19] to grow microalgae but at

different nutrient concentrations. For example, in this study, the peak point of growth is due to some nutrients optimally utilized on day 7 of cultivation.

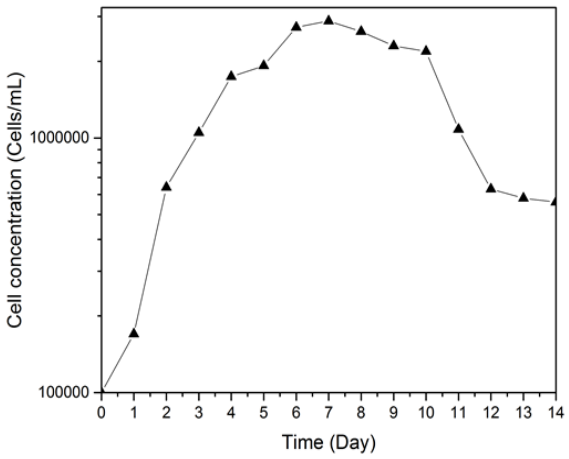


**Fig. 1** Growth of *Botryococcus* sp. in Bold Basal medium (BBM)

### 3.2 Fatty acid composition

Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs)were found to be the main constituents in the fatty acid composition of *Botryococcus* sp. gas chromatography separation determined the individual fatty acids, and 19 fatty acids were identified as shown in Fig. 1. The C4:0, C6:0, C11:0, C12:0, C14:0 C16:0, C15:1, C16:0, C16:1, C17:0, C17:1, C18:0, C18:0, C18:1n9c, C18:2n6cis, C18:3n6G, C18:3n3A, C22:2, fatty acid results were calculated as the percentage of total lipid (Table I). The major saturated fatty acids (Fig. 2 and Table 1) were Palmitic acid (C16:0) (27.950 %) and stearic acid (C18:0) (8.635%). Linoleic acid (C18:2n6cis) (17.046%), Linolenic acid (C18:3n) (22.758) and Docosadienoic acid (C22:2) (3.057) were the major polyunsaturated fatty acids. Palmitoleic acid (C16:1) (1.453%) and Oleic acid (18:1) (13.270%) were also found as high MUFAs in the *Botryococcus* sp. The results are in accordance with the studies described by [10, 20].

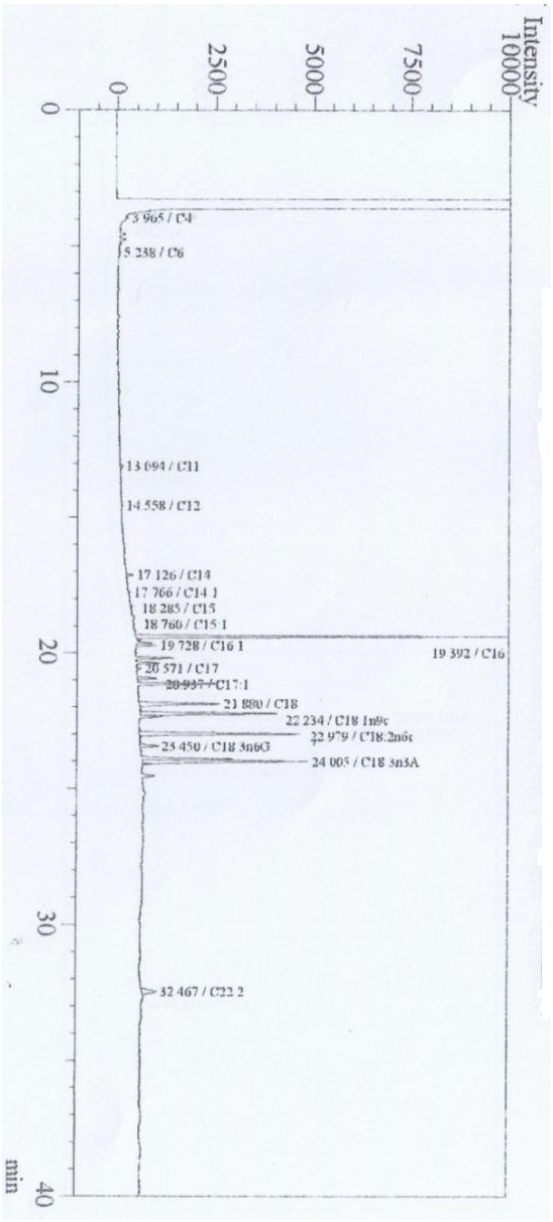


**Fig. 2** GC-MS chromatogram of *Botryococcus* sp.

This study found that the percentages of saturated and unsaturated fatty acids were 38.628% and 61.372% respectively (Table 1). The combination of C16 - C18 fatty acids alone was 95.384% of the total fatty acids; hence making the fatty acids from this microalgal strain favorable for biodiesel production. [13] also reported the highest percentage of fatty acid found in *Botryococcus* sp. to be the C16:0 fatty acid. Furthermore, the fatty acid composition of *Botryococcus* sp. observed in this study is in accordance with [11, 22].

**Table 1** Fatty acid profile of *Botryococcus* sp.

| S/N | Lipid Numbers | Common Name | % |
|---|---|---|---|
| 1 | C4 | Butyric acid | 0.255 |
| 2 | C6 | Caproic acid | 0.110 |
| 3 | C11 | Undecylic acid | 0.404 |
| 4 | C12 | Lauric acid | 0.089 |
| 5 | C14 | Myristic acid | 0.436 |
| 6 | C14:1 | Myristoleic | 0.083 |
| 7 | C15 | Pentadecylic acid | 0.129 |
| 8 | C15:1 | C15:1(*cis*10) Fatty acid | 0.051 |
| 9 | C16 | Palmitic | 27.950 |
| 10 | C16:1 | Palmitoleic | 1.453 |
| 11 | C17 | Margaric acid | 0.618 |
| 12 | C17:1 | C17:1(*cis*-10) Fatty acid | 1.532 |
| 13 | C18 | Stearic acid | 8.635 |
| 14 | C18:1n9c | Oleic acid | 13.270 |
| 16* | C18:2n6c | Linoleic | 17.046 |
| 17* | C18:3n6G | Linolenic (GLA) acid | 2.122 |
| 18* | C18:3n3A | Linolenic (ALA) acid | 22.758 |
| 19* | C22:2 | Docosadienoic acid | 3.057 |
| 20 | C16-C18 | | 95.384 |
| 21 | Saturated fatty acids | | 38.762 |
| 22 | Unsaturated fatty acids | | 61.238 |
| 23 | Monounsaturated fatty acids | | 16.723 |
| 24 | Polyunsaturated fatty acids | | 44.983 |
| 25 | Total of fatty acids | | 100 |

## 4. Conclusion

This study set out to identify the fatty acid content of local green microalga *Botryococcus* sp. cultivated using a synthetic medium. The result of this investigation shows that this indigenous *Botryococcus* sp. gave high biomass and lipid yields. Its fatty acid composition makes it a suitable feed-stock for large-scale biodiesel production. However, further investigations and experimentation into its mass production for biodiesel are strongly recommended.

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