Study on Suitable Light Conditions and Efficient Lipid Extraction Technologies for Biodiesel Production Based on Microalgae

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Abstract. As a new generation biodiesel feedstock, microalgae have most potential to replace fossil fuel. However, the limited scale and high cost are two bottleneck problems. Efficient microwave-assisted lipid extraction technologies and suitable light conditions for Chlorella Sorokiniana need further study for lowering the cost. In this study, three photoperiod groups (24L:0D, 12L:12D, 0L:24D), three illumination intensity groups (1800 lux, 3600 lux, 5400 lux), and four light spectrum groups (Red, green, blue, and white) were used to culture Chlorella Sorokiniana to investigate those effects on algae growth rate and biomass accumulation. The suitable microwave treatment was also studied to achieve an optimizing quantum fracturing technology. 400 w, 750 w and 1000 w microwave power were set and 60 °C, 75 °C, 90 °C microwave conditions were investigated. The results showed that Chlorella Sorokiniana under 24L:0D photoperiod with 5400 lux white light can achieve better growth rate. The 90 °C / 1000w microwave treatment was identified as the most simple, easy, and effective way for lipid extraction from Chlorella Sorokiniana. As the raw material of biodiesel production, C18:1, C18:2 and C18:3 have accounted for important components of fatty acid in Chlorella Sorokiniana. Therefore, Chlorella Sorokiniana is a good raw material for the production of good quality biodiesel under suitable and efficient technologies.

Keywords: Chlorella Sorokiniana; Growth rate; Light conditions; Microwave-assisted lipid extraction.

1. Introduction

Excessive consumption of non-renewable energy has resulted in global energy shortage, pollution and other issues. As a renewable clean energy, biodiesel has been drawing widespread concern in recent years. Biodiesel is a fatty acid methyl ester (FAME) obtained by the esterification of glycerol with fatty acids, products of triacylglycerol (TAG) from vegetable oil or animal oil and alcohol catalyzed by acid or alkali [1]. Early studies have shown that microalgae, kind of lower plants with high photosynthetic efficiency, environmental adaptability, short growth cycle and high biological yield, use light to product lipids efficiently and are the ideal raw material for biodiesel production [2]. The process of microalgae biodiesel production has basic steps of algae cultivation, harvesting, lipids extraction, transformation,
comprehensive product development and utilization [3]. However, scale and high cost are still two bottleneck problems in industrial application of the microalgal biodiesel production technology.

Illumination is very important for unicellular algae, and different algae have different requirements for light. In recent years, many researches on the impacts of adsorption of heavy metals and different nutrient culture conditions on microalgae growth have been reported [4-6], and yet photoperiod, illumination intensity, spectral composition and other factors still need exploration.

Traditional biodiesel extraction has many defects, including long time extraction, large solvent demand, and high energy consumption etc. If these defects could be solved, biodiesel would become more economical and environmentally-friendly [7]. A new technology, microwave pretreatment, has been developed in recent years. It has been applied to the extraction of tea polyphenols, plant polysaccharides and other natural products, and increased the yield significantly [8]. We supposed that it was considered feasible to apply microwave pretreatment technology to biodiesel production to extract lipid effectively from microalgae.

In this study, we explored the appropriate light conditions for the growth of Chlorella Sorokiniana, and suitable microwave pretreatment methods to enhance methyl ester yield. And these results may help to provide the foundation for large-scale microalgae cultivation and biodiesel production.

2. Materials and Methods

2.1. Materials

Chlorella Sorokiniana was obtained from Shenzhen engineering laboratory for algal biofuel technology development and application, Peking University.

2.2. Instruments

Illumination incubator (Yiheng, MGC-450HP-2, China), high speed freezing centrifuge (Hettich UNIVERSAL 320R, Germany), high speed centrifuge (Eppendorf 5418R, Germany), microwave-assisted extraction system (Milestone Ethos, Italy), freeze drier (LabconcoFreeZone 2.5, US), and spectrometer (Shimadzu, Japan).

2.3. Culture Medium

The cultures were supplied with BG-11 medium.

2.4. Algae Cells Calculation

Algae sample was diluted to 9 gradients. Cells were counted by haemacytometer and optical density was measured by spectrometer at 685 nm wavelength. Standard curve and trend line was generated for further cell numbers calculation

2.5. Chlorophyll a Content Calculation

1 ml algae sample was centrifuged at 13000 rpm for 10 min. Supernate was discarded and 1 ml 90% methanol was added. After 50 °C water bath for 1 hour, the samples were centrifuged at 13000 rpm for 10 min. Supernate was transferred into a new tube and the absorbance at 665nm and 750 nm were measured by spectrometer. The chlorophyll a content was calculated by the followed formula:

\[
\text{chlorophyll a} = \frac{139 \cdot (A_{665} - A_{750}) \cdot V_{\text{methanol}}}{V_{\text{sample}} \cdot \text{L}} \text{ (\text{\mu g} \cdot \text{mL}^{-1})}
\]

where \(V_{\text{methanol}}\) is the methanol volume (ml), \(V_{\text{sample}}\) is the sample volume (ml) and \(\text{L}\) is the width of cuvette (cm)

3. Different Photoperiods, Illumination Intensities and Light Spectrums Cultivation

600 ml algae was aerobic cultured in 1 L erlenmeyer flask in an illumination incubator at 24 °C. The cultivation condition is shown in table 1.
Table 1. Cultivation Conditions for the experiments on different photoperiods, illumination intensities and light spectrums.

| Groups      | Photoperiod | Illumination intensity | Light Spectrum       |
|-------------|-------------|------------------------|----------------------|
| Photoperiod | 24L:0D      | 5400lux                | White light          |
|             | 0L:24D      | 5400lux                | White light          |
|             | 12L:12D     | 5400lux                | White light          |
|             | 24L:0D      | 1800lux                | White light          |
| Illumination intensity | 24L:0D      | 3600lux                | White light          |
|             | 24L:0D      | 5400lux                | White light          |
|             | 24L:0D      | 3600lux                | Red light(650~760nm) |
| Light Spectrum | 24L:0D      | 3600lux                | Green light(500~560nm) |
|             | 24L:0D      | 3600lux                | Blue light(430~470nm) |
|             | 24L:0D      | 3600lux                | White light(390~760nm) |

Note: Photoperiod “12L: 12D” refer to 12hrs. Light (L) culture time and 12hrs. Dark (D) culture time by turns.

3.1. Chlorella Sorokiniana Scale Cultivation and Harvest

Chlorella Sorokiniana was outdoor large scale cultured in a 60 L incubator at 27-35 °C. The algae were harvested after 10-day cultivation. Dry biomass was obtained using a freeze drier.

3.2. Microwave-assisted Extraction

One-step lipid extraction and transesterification method combines the algal lipid extraction process and lipid transesterification process together so that the FAME yield (FAME weight / dry biomass of algae) from dry biomass can be tested by GC (gas chromatography) system directly. A microwave-assisted extraction experiment was constructed to treat on algae cells for higher yield. The power of microwave 400 W, 750 W, 1000 W and the temperature 60 °C, 75 °C, 90 °C were designed as a cross over experiment for the study of different power and temperature of microwave treatment.

3.3. Composition Analysis

For the quantification of reaction product, the algal biodiesel samples were analyzed by GC system incorporated with an Agilent 7890 AGC equipped with a capillary column DB-5ms (60 mx250 μm×0.25 μm).

4. Results

4.1. Cell Number and Chlorophyll a Content under Different Photoperiods

There was a significant difference in microalgae growth at 24L:0D, 12L:12D and 0L:24D (Fig. 1). Three groups, 24L:0D, 12L:12D and 0L:24D had the same initial inoculum density (3.99×10^5 cells•ml^-1) and the same chlorophyll a content (0.241 mg•ml^-1). Compared with 24L:0D group, the cell number and chlorophyll a content of Chlorella Sorokiniana from 12L:12D group were obvious lower, and the algae under 0L:24D photoperiod condition did not grow (Fig. 1 and 2).
Figure 1. The growth curves of Chlorella Sorkiniana under different photoperiods.

Figure 2. The Chlorophyll a content of Chlorella Sorkiniana under different photoperiods.

4.2. Cell Number and Chlorophyll a Content under Different Illumination Intensities
The cell number for 5400 lux was higher than those for 3600 lux and 1800 lux (Fig. 3). The highest illumination intensity gave the highest cell density (2.448×10⁷ cells•ml⁻¹) and chlorophyll a content (Fig. 4) in this research.

Figure 3. The growth curves of Chlorella Sorkiniana under different illumination intensities.
4.3. Cell Number and Chlorophyll a Content under Different Light Spectrums
In the 7th day, the cell densities (from high to low) under different light spectrums were \(2.5608 \times 10^7\) cells\(\cdot\)ml\(^{-1}\) (White light), \(2.0895 \times 10^7\) cells\(\cdot\)ml\(^{-1}\) (Blue light), \(1.5828 \times 10^7\) cells\(\cdot\)ml\(^{-1}\) (Red light) and \(1.4337 \times 10^7\) cells\(\cdot\)ml\(^{-1}\) (Green light). Chlorophyll a contents from high to low were 20.87 mg •L\(^{-1}\) (White light), 17.57 mg •L\(^{-1}\) (Blue light), 15.78 mg •L\(^{-1}\) (Blue light) and 13.14 mg •L\(^{-1}\) (Green light).

**Figure 4.** The Chlorophyll a content of *Chlorella Sorkiniana* under different illumination intensities

**Figure 5.** The growth curve of *Chlorella Sorkiniana* under different light spectrums

**Figure 6.** The Chlorophyll a content of *Chlorella Sorkiniana* under different light spectrums.
4.4. FAME Yield under Different Microwave Pretreatment Times

The FAME yield is 9.02% by one-step lipid extraction and transesterification method without microwave pretreatment (Fig. 3). Using microwave to treat the algae for 2 min and 5 min at 700 w increased the yield by 2.4%. Yet the difference between 5 min treatment (11.41%) and 7 min (11.48%) treatment was not obvious.

![Figure 7](image1.png)

**Figure 7.** The FAME yield according to different microwave pretreatment times

4.5. FAME Yield under Different Microwave Power and Different Temperature

With changing Chlorella microwave pretreatment power and target temperature, FAME yield has changed significantly (Fig. 8). Increasing the microwave power and temperature significantly promoted the FAME yield. The FAME yields with 400 W, 700 W and 1000 W microwave pretreatment at 60 °C were 11.75%, 12.36% and 12.86%, respectively. Compared to 60 °C, the FAME yield (13.42%) increased significantly at 90 °C for 1000 W microwave pretreatment.

![Figure 8](image2.png)

**Figure 8.** The FAME yield according to different microwave temperature and power

4.6. Fatty Acid Composition

Different cell disruption methods had no obvious effects on the major fatty acid composition of *Chlorella Sorokiniana*. As the raw material of biodiesel production, C16:0 (20.78%), C18:0 (7.87%), C18:1 (13.45%), C18:2 (20.59%) and C18:3 (22.93%) are the dominant components of fatty acid in *Chlorella Sorokiniana*. Therefore, fatty acids from *Chlorella Sorokiniana* were suitable for the production of biodiesel.

5. Discussion

The experimental results showed that *Chlorella Sorokiniana* had a high demand for photoperiod. Algal cells can grow better with the prolongation of photoperiod. The blank control group demonstrated that
Chlorella could not grow without light. According to De-Bashan et al. [9], *Chlorella Sorokiniana*, with high temperature and illumination intensity tolerance, can be used in extreme hot desert areas for sewage treatment. Qiao and Wang [10] reported that *Chlorella Sorokiniana* could grow at 30 °C, and could withstand 4000 lux continuous light. In the study of *Alexandrium* and *Scrippsiella trochoidea*, Deng et al. [11] found that biomass accumulation of the two algae increased significantly under continuous light. Chen et al. [12] pointed out that the optimal illumination range of algae was between 2000 ~ 10000 lux, and more than 10,000 lux illumination would lead to light suppression. Richmond et al. [13] also found that Chlorella had illumination intensity tolerance and more than a certain illumination culture Chlorella would have light suppression, which would reduce the Chlorella yield. Chlorella pigment composition is similar to both *Alexandrium* and *Scrippsiella trochoidea*. Their growth patterns are also similar. Lu et al. [14] found that in three different kinds of photoperiod (6L: 18D, 12L: 12D and 18L: 6D), Chlorella sp. grew best in 12L:12D. However, our experiment results showed that compared to 12L: 12D photoperiod, Chlorella in 24L: 0D photoperiod grew better, which indicated that a short period continuous illumination without exceeding a certain intensity could promote Chlorella growth.

In similar experimental conditions, our experiment drew the similar conclusion with other scholars. The density of algal cells can be reflected by the chlorophyll content in the unit water [15]. Our results showed that in the range of 1800 - 5400 lux, the algal cell had the greatest density and chlorophyll a content under 5400 lux. Yan et al. [16] studied the effects of light on the growth rate and chlorophyll content of Chlorella and Scenedesmus, and found that the optimum illumination intensity for Chlorella was 5000 lux. Xu et al. [17] used the same culture medium and similar conditions to figure out the optimum illumination intensity range of Chlorella growth was between 5000 lux and 5500 lux. Ouyang et al. [18] found that the illumination intensity determined the algae photosynthesis rate with certain temperature, pH and nutrition. Before the light saturation point, the greater the illumination intensity is, the higher the algae photosynthesis rate will be.

Our experiments indicated that *Chlorella Sorokiniana* had the best growth in white light, followed by blue, red, and finally green. As for monochromatic lights, blue light was more beneficial to *Chlorella Sorokiniana* growth than red light. According to Das et al. [19], blue light is more beneficial to the growth of Nannochloropsis sp. in Chlorophyta both autotrophically and heterotrophically. Chlorella, containing both chlorophyll a and chlorophyll b, has a wide absorption band in both red and blue light. White light is more suitable for Chlorella growth than either blue or red light, because white light includes both blue and red light. From the perspective of evolution, Chlorella lives near the surface of seawater, in which the component of white light changes insignificantly, so Chlorella could adapt to white light well.

In biodiesel production, the FAMEs are mainly obtained by transesterification of fatty acids with a chain length of C12 to C20. In the fatty acid components of *Chlorella vulgaris*, C18: 1, C18: 2, and C18: 3, three unsaturated fatty acids, account for the most important ingredients, which indicates that Chlorella has the potential for biodiesel production.

According to our results, microwave pretreatment could increase the yield of biodiesel. Heat can be directly conducted to highly polar substances by microwave with high thermal efficiency through molecular polarization or ion conduction [20]. After microwave treatment, cell wall of algal cells will be damaged, which makes the extraction solvent access to algae cells more easily, and advances extraction efficiency. Progress has been made in microwave process study in order to increase target products yield, such as Trehalose [21] and Artemisinin [22]. Zheng et al. [23] indicated that microwave treatment could increase the yield of fatty acids through treating *C. vulgaris* by different methods. Balasubramanian et al. [24] found that high temperature conditions can achieve higher yields in microwave treatment of algae. Cravotto et al. [25] treated soybean oil with the 2450 MHz microwave system for half an hour, and found that the amount of extracted vegetable oil was 2 times more than that with conventional Soxhlet method for 4 hours. According to Zhang [26], the oil content (dry biomass) of Chlorella sp. was approximately 30%. Our results showed that the lipid yield (lipid / dry biomass) of *Chlorella Sorokiniana* increased from 9.02% to 13.42% by 4.4 percentage points, after microwave treatment with high power and high temperature. It’s conceivable that the extraction rate (lipid yield / oil content) increased from 30.1% to 44.7% by 14.6 percentage points, which was 1.5 times of the original yield. The
significant increase of the extraction rate compared to the original indicates that microwave treatment is feasible. And microwave treatment in different conditions did not change the fatty acid composition of *Chlorella Sorokiniana* significantly, which illustrated that this experimental program could be used to produce biodiesel.

6. Conclusion
This study, finding the suitable light conditions of *Chlorella Sorokiniana* and the microwave extraction method of lipid, will benefit the further development of microalgae bioenergy. The study showed that *Chlorella Sorokiniana* could achieve higher growth rate under 24L:0D photoperiod with 5400 lux white light. White light could promote *Chlorella Sorokiniana* growth most significantly, followed by blue, red, and finally green. In addition, compared with 400 W / 60 °C, the FAME yield increased by 14% for the microwave pretreatment at 1000 W / 90 °C. Therefore the 90 °C / 1000 w microwave method was identified as the most simple, easy, and effective way for lipid extraction from *Chlorella Sorokiniana*. As the raw material of biodiesel production, C18:1, C18:2 and C18:3 have accounted for important components of fatty acid in *Chlorella Sorokiniana*. Therefore, *Chlorella Sorokiniana* could be a good raw material for the production of good quality biodiesel with optimizing light condition and lipid extraction method.

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