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# Scale-up cultivation of *Chlorella ellipsoidea* from indoor to outdoor in bubble column bioreactors

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## HIGHLIGHTS

- *Chlorella ellipsoidea* cells were cultivated in bubble column bioreactors.
- Microalgal cells were able to quickly adapt to the outdoor conditions.
- Biomass production cost in outdoor culture was lower than that in indoor culture.

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## ABSTRACT

The cultivation of *Chlorella ellipsoidea* in bubble column bioreactors was investigated at different scales under indoor and outdoor conditions. The algal cells were able to quickly adapt to the outdoor conditions and achieved a growth rate of 31.55 mg $L^{-1}$ $day^{-1}$. Due to differences in light and temperature, the outdoor culture produced a higher percentage of unsaturated fatty acids compared to the indoor cultures, while the amino acid composition was unaffected. The overall cost of the biomass produced by the 200 L outdoor cultivation (58.70 US$/kg-dry weight) was estimated to be more than 7 times lower than that of the 20 L indoor cultivation (431.39 US$/kg-dry weight). Together these results provide a basis for the cultivation of *C. ellipsoidea* for the large-scale production of biofuels, high-value nutrients and/or recombinant proteins.



## 1. Introduction

Microalgae are photoautotrophic sunlight-driven cell factories that can convert carbon dioxide to various products such as lipids, carbohydrates, proteins, fatty acids, vitamins, antibiotics, and antioxidants (Chisti, 2007). In addition to these naturally-produced compounds, microalgae are also a promising platform for the production of recombinant proteins. Microalgae have unique advantages, including a rapid growth rate, ease of cultivation, and the ability to make the same post-transcriptional and post-translational modifications as plants (Potvin and Zhang, 2010). The feasibility of utilizing microalgae as a production system for therapeutic or industrial proteins has been previously demonstrated (Specht et al., 2010). *Chlorella ellipsoidea* is a single-celled eukaryotic green algae that has been extensively studied. As with other *Chlorella* sp, it can be found in both fresh and marine water systems, and it can be cultured under autotrophic, heterotrophic, mixotrophic, or photoheterotrophic growth conditions (Mata et al., 2010). It is rich in high-quality proteins, vitamins, lipid-soluble compounds, glycolipids, sulfolipids, and compounds used as food additives. It is also beneficial for human health due to its ability to lower blood sugar levels and increase hemoglobin concentrations, and it is used to enhance animal growth when it is applied as an aquaculture or animal feed (Mata et al., 2010; Kay, 1991). Furthermore, *C. ellipsoidea* is a well-established model organism and a promising bioreactor for the production of complex foreign proteins for pharmaceutical and industrial use (Wang et al., 2003; Walker et al., 2005). The genetic transformation of *C. ellipsoidea* is feasible, and it has been previously utilized for the recombinant production of flounder growth hormone (FGH) and rabbit neutrophil peptide-1 (NP-1) (Liu et al., 2013; Kim et al., 2002; Chen et al., 2001; Bai et al., 2013).

For the industrial production of products from microalgae, it is necessary to evaluate the feasibility of scaled-up outdoor culture systems. The utilization of natural sunlight and uncontrolled

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outdoor culture conditions can reduce the overall costs of the cultivation process (Feng et al., 2011). Raceway ponds and other open-culture systems are commonly used for large-scale outdoor cultivation due to their low costs for construction and operation; however, they often perform poorly due to contamination risk, lack of control of the culture conditions, and problems with mixing and light utilization efficiency (Mata et al., 2010). Closed bioreactors can provide a suitable environment in terms of light, nutrients, $CO_2$, and temperature, and they have the potential to produce higher rates of biomass production and improved culture quality (Brennan and Owende, 2010). The feasibility of *Chlorella* sp. outdoor cultivation has been confirmed by many studies using different types of bioreactors at scales ranging from 10 to 70 L, including a flat glass plate bioreactor, a cylindrical glass bottle bioreactor, and a nylon membrane column bioreactor. The results have demonstrated that many *Chlorella* strains can adapt to the fluctuating temperatures and irradiance levels inherent in outdoor cultivation, and that these systems can be used for biodiesel production (Feng et al., 2011, 2012; Zhou et al., 2013). In addition, the outdoor production of *Chlorella* sp. can also utilize the waste produced by other processes for nutrients, such as organic fertilizer derived from food waste or manure, dairy and piggery wastewater, or flue gas generated by the combustion of natural gas (Chisti, 2013). This can decrease the cost of the cultivation process (Doucha et al., 2005; Lam and Lee, 2012; Huo et al., 2012; Zhu et al., 2013). However, the growth rate is often reduced by outdoor cultivation; for example, the growth rate of *Calluna vulgaris* was reduced by 27% in a study by Lam and Lee comparing outdoor cultivation with cultivation in a controlled environment (Lam and Lee, 2012). In addition, the lipid content and fatty acid composition also varied in response to climatic variation (Olofsson et al., 2012), which may influence the quality of the final products.

Although there are numerous reports describing the outdoor cultivation of microalgae, studies focusing on *C. ellipsoidea* are rare and the culture scale in published studies is limited. It is important to understand the variations in growth and metabolism between indoor and outdoor cultivation conditions in order to determine the feasibility of outdoor cultivation for this species. In the current study, a 200 L outdoor cultivation system was constructed using a bubble column bioreactor made of a polyethylene membrane. The cultivation of *C. ellipsoidea* in bubble column bioreactors was scaled-up from the 20 L indoor culture to a 200 L outdoor culture, and the biomass, fatty acid content and composition, protein content, and amino acid composition were investigated and compared with that of 2 L sterilized flanged glass bioreactor. The effect of controlled and uncontrolled light intensity and temperature on *C. ellipsoidea*'s growth and metabolism was analyzed, and the cost of each cultivation system was calculated.

## 2. Methods

### 2.1. Microalgae strain and culture systems

*C. ellipsoidea* (UTEX 20), kindly provided by Prof. Zan-Min Hu (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences), was grown on BG-11 medium. The cells were maintained in 250 mL Erlenmeyer flasks containing 100 mL liquid medium and incubated at 25 °C in an orbital shaker at 100 rpm. Algal cells were grown photoautotrophically under a 16 h light period per day with 35 μmol $m^{-2}$ $s^{-1}$ for 15 days. The pre-cultured cells were used as the inoculum for all bubble-column cultivation experiments.

The 2 L bubble column bioreactor was constructed from a flanged glass column (Beijing Glass Group Company, Beijing, China), and the 20 and 200 L bubble column bioreactors were constructed from transparent polyethylene (PE) bag with a thickness of 0.2 mm (Yangpu Packaging Material Co., Ltd., Hebei, China). The bottom of the plastic culture bags with the desired dimensions was heat-sealed by a SF-B 800 pedal sealing machine (Xingye Machinery Equipment Co., Ltd., Wenzhou, China). The size of all bioreactors was shown in Supplementary material-Table 1. Air was supplied using an air sparger (compressed stainless steel particles) at the bottom of each bioreactor. Each 20 L bioreactor was handed at a shelf, and each 200 L bioreactor was supported by a circular wire mesh.

The indoor cultivations were performed at 25 °C under a light/dark cycle of 16/8 h with 25,000 lux illumination intensity, and the outdoor cultivations were conducted under the natural temperature and light conditions in Haidian district of Beijing, China (latitude 39°59′ N, longitude 116°19′ E), in autumn (September 29th, 2012 to October 19th, 2012). The initial biomass concentration was 0.075 g/L, and $CO_2$-enriched air (1%) was bubbled into the reactor at a flow rate of 0.1 v/v/min through a 0.22 μm filter (Millipore, MA, USA) for the 2 L indoor cultivation and directly aerated into the reactor for the 20 L indoor and 200 L outdoor cultures. For the 2 L indoor culture, the reactor and the air supply systems were sterilized by autoclaving at 121 °C for 40 min prior to starting the cultivation.

### 2.2. Microalgae growth measurement

To determine the relationship between the optical density at 680 nm ($OD_{680}$) and the algal biomass (g/L), the algal culture broth was diluted or concentrated to a certain $OD_{680}$, then harvested by centrifugation for 5 min at 10,000 rpm, 4 °C. The algal pellets were then washed three times with distilled water and dried at 105 °C to a constant weight. The dry weight (DW, g/L) of the algal cells was determined gravimetrically. The relationship between the $OD_{680}$ and the *C. ellipsoidea* biomass was as follows:

$$\mathrm{DW(g/L)} = 0.38352 \times \mathrm{OD_{680}} - 0.02647 \ (R^2 = 0.9996) \quad (1)$$

The algal growth was measured every two days by measuring the $OD_{680}$ of the culture broth using a UV-2100 spectrophotometer (Unico, Shanghai, China) and the biomass was calculated using Eq. (1).

The maximum specific growth rate ($\mu_{max}$, $day^{-1}$) at the exponential stage was calculated as follows:

$$\mu_{\max}(\mathrm{day}^{-1}) = (\ln \mathrm{DW}_2 - \ln \mathrm{DW}_1)/(t_2 - t_1) \quad (2)$$

where $DW_1$ and $DW_2$ were the dry biomass weight (g/L) at time $t_1$ and $t_2$, respectively.

The doubling time ($T_D$, days) was calculated as follows:

$$T_D(\mathrm{days}) = \ln(2)/\mu_{\max} \quad (3)$$

The rate of biomass production ($P$, mg $L^{-1}$ $day^{-1}$) was calculated according to the following equation:

$$P(\mathrm{mg\ L^{-1}\ day^{-1}}) = (\mathrm{DW}_x - \mathrm{DW}_0)/t_x \quad (4)$$

where $DW_0$ and $DW_x$ were the initial dry biomass weight (mg/L) and the dry biomass weight (mg/L) at time $t_x$, respectively.

### 2.3. Lipid analysis

For the extraction and transesterification of the fatty acids, 50 mg lyophilized algal biomass was ground for 10 min and then dispersed in 3 mL of a 7.5% (w/v) $KOH/CH_3OH$ solution with 200 mg of heptadecanoic acid added as an internal standard. After the solution was incubated at 70 °C for 4 h, 2 mL $HCl/CH_3OH$ (1:1, v/v) and 2 mL 14% $BF_3/CH_3OH$ (ANPEL, Shanghai, China) were