Optimization of medium for enhanced biomass and lipid production in *Elliptochloris subsphaerica* through mixotrophic cultivation

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Introduction

In response to the energy crisis driven by climate change, research has shifted from fossil fuels to biofuels for their renewability. As a result, microalgae have emerged as a promising resource for biofuel due to their high efficiency of lipid production. Among different cultivation methods, mixotrophic cultivation, which integrates autotrophic and heterotrophic growth, has demonstrated the ability to enhance biomass and lipid productivity, making it suitable for large-scale production.

Botryococcus braunii is one of the most researched species for the development of biofuels, renowned for its high hydrocarbon production, reaching up to 86% of its weight (Borowitzka, 2018). However, its slow growth rate increases the cost of production. To address this, *Elliptochloris subsphaerica*, a green algal species closely related to *B. braunii* (Fučíková et al., 2014), is being explored for its potential.

E. subsphaerica is known to exist as a phycobiont in lichen (Masumoto, 2020) as well as free-living in various substrates (Veselá et al., 2024). This species has been observed to contain significant lipid droplets within its cells. A previous study has shown that *E. subsphaerica* exhibits rapid growth when cultured in medium Y, achieving a dry cell weight (DCW) of 3.5 g/L in 14 days and lipid content 30% of its DCW (Yuzheng, 2021). However, a following study has found that glucose concentration is not significant for the growth of *E. subsphaerica* in medium Y (Polsilapa, 2023).

Thus, this experiment aims to determine the optimal concentration of components X in medium Y to maximize biomass and lipid accumulation in *E. subsphaerica*.

Material & Methods

1. Medium Preparation: Four media Y (T1, T2, T3, and T4) were made with varying concentrations of components X.

2. Cultivation: Pre-culture cells were inoculated at an initial density of $5x10^6$ cells/mL and maintained at 20°C with a light/dark cycle of 14/10 h, under continuous shaking at 100 rpm.

3. Cell growth and harvest: Cell density was monitored every two days through a hemocytometer (C-chip) cell counting. Cells were harvested on day 14 and stored at -80°C before being freeze-dried for DCW.

4. Lipid staining and extraction: Cells were stained with Nile Red to assess lipid content. Lipids were extracted from dried samples using a chloroform/methanol (2:1) solution.

5. Statistical analysis: The experiments were conducted in triplicate. GraphPad Prism software was used to analyze the data using ANOVA, followed by Tukey's post hoc test.

Results and Discussion

1. Growth characteristics

E. subsphaerica grown in T4 medium showed the highest growth ($p<0.0001$), achieving a DCW of 10.07 g/L in 14 days with a productivity rate of 0.68 g/L/day. Compared to the previous experiment by Yuzheng (2021), the DCW in this study is nearly three times higher than the mixotrophic culture using medium Y with glucose. These results suggest that higher components X concentrations promote greater growth by providing more nutrients, allowing algae to extend the growth period before reaching the stationary phase. Further study is needed to determine the peak of growth by extending the experiment period and testing more treatments. Additionally, the cell size in T4 medium reached the largest diameter on day 14, measuring twice the diameter of cells on day 0, with large lipid droplets.

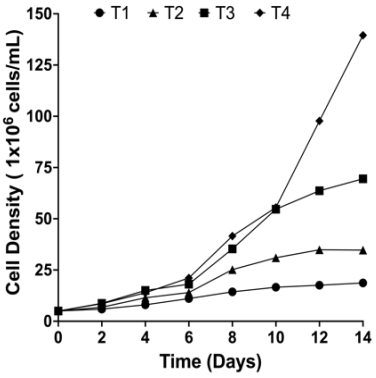

Fig.1 Cell density of *E.subsphaerica* grown in different medium concentration in 14 days

2. Lipid contents

Fluorescent microscope observation of cells stained with Nile Red showed bright stained lipid droplets of algae grown in all media. Subsequent lipid extraction showed that algae grown in T4 medium had the highest lipid content, with 37.6% of its DCW as lipids ($p<0.0001$). This amount significantly surpasses the previous study by Yuzheng (2021), which reported a lipid content of 30% of DCW. These findings suggest that higher components X concentrations not only enhance growth but also significantly increase lipid accumulation in *E. subsphaerica*, making it a viable candidate for sustainable oil production. Further lipid content identification is needed in the future to enhance its potential as a valuable source of algal oil.

References

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