Optimisation of Glucose Concentration in Mixotrophic Cultivations of a Lichen Phycobiont *Ellipitochloris subphaerica*

Tawan Polsilapa （筑波大学 生物学類）　　指導教員：Ishida Ken-Ichiro (筑波大学　生命環境系)

1. Introduction

The transition towards utilizing renewable resources for bioenergy has sparked considerable interest, with a particular focus on microalgae known for their high lipid content. Among microalgae, *Botryococcus braunii,* a trebouxiophycean green alga, is one of such microalgae and known for its high production of extracellular long-chain hydrocarbons, comprising up to 86% of its total dry weight (Borowitzka, 2018). However, *B. braunii* grow very slowly in culture, which raises the cost of hydrocarbon production.

Elliptochloris subsphaerica, a close relative of *B. braunii*, is a phycobiont of a lichen. In a previous study conducted at the laboratory to which I am affiliated, *E. subsphaerica* exhibited fast growth (3.5 g L^{-1} DCW in 14 days) and significant oil accumulation (30% of DCW) when cultured in medium X with 2% glucose (Shen, 2021). However, *E. subsphaerica* did not thrive in GTY medium, also containing 2% glucose (Shen, 2021), prompting questions about the role of glucose in its cultivation.

This experiment aims to identify the best glucose concentration for cultivating *E. subphaerica* with high biomass and lipid ratio. The effects of glucose concentrations in medium X ranging from 0% to 4% on growth characteristics and lipid accumulation were assessed.

2. Material & Methods

2.1. Culture media & Conditions: Five medium X with glucose concentrations of 0, 10, 20, 30, & 40 g L^{-1} each, along with a constant rate of other substances were prepared. Cells were inoculated at a concentration of $1x10^6$ cells per *mL,* under the light intensity at 250μ *mol* m^{-2} s^{-1} at 21°*C*, 100 rpm.

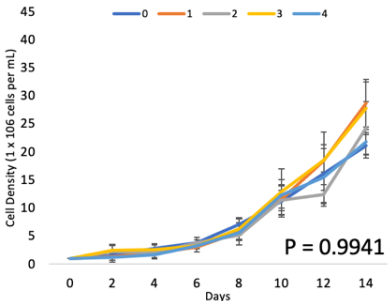
2.2. Cell Growth: The cellular growth was monitored biweekly, with observations taken once every two days via manual cell count by C-Chip (F01). Dry Cell Weight (DCW) was measured at the start and end of each experiment. Cells were harvested by centrifugation, froze at -80°*C*, and freeze-dried overnight.

2.3. Statistical Analysis: The analysis of data was carried out by Prism 9 software. The statistical significance was determined by analysis of variance (ANOVA). A P value < 0.05 was considered as statistically significant.

2.4. Observation of Lipid Accumulation: Cells collected on day 14 were stained with Nile-red dye at a ratio of 1:100 and observed under a fluorescent microscope.

3. Results & Discussion

3.1. Growth Characteristics: *E. subphaerica* did not present any significant differences in growth rate among the conditions, Fig 1 (p = 0.8912 - 0.9941). Mixotrophically cultured green algae such as *Chlorella pyrenoidosa* has been known to utilise monosaccharides such as glucose for their growth (Zhang el. al., 2014). On the contrary, my result indicates that *E. subphaerica* is not able to use glucose in mixotrophic condition but utilises alternative substances as its carbon source in the medium X, which differs from many previous reports. Moreover, *E. subsphaerica* achieved a high biomass yield (2.55 - 3.94 g L^{-1} DCW) and cell density (27.2 million cells mL^{-1}) over two weeks using a glucose-free medium. This suggests potential cost reduction in large-scale cultivation of *E. subsphaerica* for bioenergy, reducing the dependence on glucose.



3.2. Lipid Analysis:

Lipid droplets (LD) were observed in all conditions, with no significant difference (p > 0.9999) related to growth rate. Optimising LD accumulation and identifying unknown lipid constituents are crucial for utilizing *E. subphaerica* as a viable resource for oil production. If the significant portion of the unidentified accumulated lipid (approximately 80%) hydrocarbons (Shen, 2021), it would further enhance its potential as a valuable source for hydrocarbon production.

References

Borowitzka, M. A. (2018). Biology of microalgae. 23–72

Zhang et al. (2014). Bioresource Technology. 173, 52–58

Shen. Y. (2021). Tsukuba Journal of Biology.