Optimization of the environmental factors for the growth and biomass production of the green alga, *Monoraphidum neglectum*

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Introduction

Biofuel is one of the hot candidates which can remedy lots of environmental issues caused by excess consumption of the fossil fuels. However, since the biofuel is predominantly made by edible crops such as maize or soybean nowadays, it accelerates shortage in foods as well. In order to solve this issue, microalgae are considered as candidates of a sustainable production host for biofuel and other biotic products (Bogen et al. 2013). Among lots of different species, freshwater microalgae *Monoraphidium neglectum* shows a high potential for biofuel production as well as growth in a wide range of pH and temperature, which can be utilized in the field of bioremediation as well. In this research, the optimal conditions for the growth of *M. neglectum* were evaluated in terms of temperature, pH and compositional factors in growth media.

Materials and Methods

M. neglectum A11 strain was pre-cultivated for 2 d with starting optical density (OD) 0.2, and then cultivated up to 8 d. BG-11 was selected as the standard medium. The standard growth conditions were continuously illumination with 70 µmol photons/m^2/s of white light and aerated with 1% (v/v) CO_2 enriched air. The growth temperature was set to 25°C except temperature tests.

The temperature test was conducted with 3 replicates per each temperature, from 10°C to 40°C with 5°C interval. For pH tests, 20 mM MES, HEPES, CHES and CAPS were used to adjust pH at 5.5, 7.5, 9.5, and 11.1, respectively, in order to minimize any drastic changes of pH during cultivation.

Compositional analysis was based on the Ilavarasi's research (2011), where Half-strength Chu 10 media (HC-10) showed a significantly higher growth rate than BG-11 for *M. neglectum* cultivation. Total of 4 different BG-11 media were tested based on the major differences between HC-10 and BG-11 media; a medium with low nitrogen content, a medium with Vitamin B_1 or SeO_3^{2-}/SiO_3^{2-}, and normal BG-11. OD at 730 nm was used to measure the growth rate of *M. neglectum*, and total amount of chlorophyll *a* and *b* was calculated based on the OD at 665 nm and 650 nm (OD at 750 nm was used for calibration). To analyse fatty acid composition and quantification of them, the total lipids were extracted by hexane and methyl-esterified fatty acids were applied on a gas chromatography.

Results and Discussion

The cells of *M. neglectum* were able to maintain its growth in the range from 10°C to 35°C for 8 d cultivation. The growth rate started to increase drastically between 15-20°C, but most cells were dead at 40°C. The highest growth rate was reached around 25~30°C in terms of both OD and chlorophyll (Fig. 1).

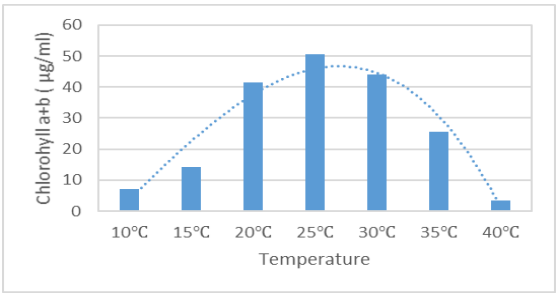

Fig 1. Chlorophyll content of *M. neglectum* in different temperatures after 3 d of cultivation

For pH tests, *M. neglectum* tended to grow much better at basic conditions than acidic conditions. The pH of media was converged to slightly basic condition (pH 8.1~8.5) during cultivation, and the growth rate was increased with this up-shift in pH. Hence, it is assumed that this species might adjust unfavorable pH conditions to increase its growth rate.

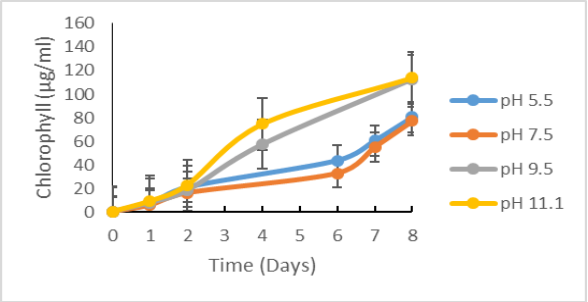

Fig 2. Chlorophyll content of *M. neglectum.* with different pH

In the media comparison tests, an addition of SeO_3^{2-}/SiO_3^{2-} showed the highest growth rate, while low N media showed the lowest result. The GC analysis of *M. neglectum* showed that it produced mainly C16:1 (24.1±1.2%), C18:1 (15.1±0.7%), C18:2 (27.9±0.8%) and C20:1 (32.9±1.2%) fatty acids. However, there are some minor peaks corresponding to unidentified fatty acids to be examined.

References

Bogen C. et al. (2013) *BMC Genomics* **14,** 926.

Ilavarasi A. et al. (2011) *Biotechnology* **10,** 540-545.