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Strategies to improve oil/lipid production of microalgae in outdoor cultivation using vertical tubular-type photobioreactors

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Abstract

The indoor laboratory-scale microalgae culture was elevated to 50 liter in outdoor photobioreactors for the cultivation of an oil-rich microalga, *Chlorella vulgaris* ESP-31. Under phototrophic and photoheterotrophic conditions using CO_2 and acetic acid as carbon source, respectively, the lipid productivity and lipid content obtained were 30-31 mg/L/d and 35-44%, respectively. The effect of inoculum sizes on the performance of biomass and lipid production under phototrophic cultivation was also investigated. The lipid productivity of *C. vulgaris* ESP-31 was further improved to 47.6 mg/L/d when a higher inoculum size of 0.70 g/L was used. Finally, to avoid severe contamination problem during outdoor cultivation in the summer time, a two-stage lipid accumulation strategy was employed. The results show that the proposed two-stage process was able to avoid the bacterial contamination and enhanced the lipid content and lipid productivity of *Chlorella vulgaris* ESP-31.

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Keywords: *Chlorella vulgaris*, tubular-type PBR, microalgae, lipids

1.Introduction

Most studies on microalgal oil production were conducted in a small scale in the laboratory (Ruangsomboon, 2012). The results of those studies cannot clearly represent the real situation when the microalgae are cultivated outdoors for the mass production of microalgal oil. In addition, the problems associated with outdoor cultivation, such as variations in light intensity, temperature, and contamination of bacterial or alien microalgal species (Doucha & Livansky, 2009). To accuractely assess the fesibility of oil-producing microalgal strains in practical applications, large-scale outdoor cultivation of the microalgae should be conducted. In this work, an oil-rich microalga, *Chlorella vulgaris* ESP-31, was grown outdoors using vertical tubular-type photobioreactor (PBR) system (50 L working volume), which is known of great benefit for photoautotrophic growth due to better CO_2 dissolution and retention. The difficulties involved in

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outdoor cultivation, such as temperature variations, inconsistent sunlight supply, contamination, and inefficient mixing. All these factors are investigated and addressed in this study. The goal of this study is to develop feasible strategies leading to enhanced microalgal lipid production in outdoor cultivation.

2. Cultivation of *Chlorella vulgaris* ESP-31 in outdoor 50 liter vertical tubular-type photobioreactor under phototrophic and photoheterotrophic conditions

Vertical tubular-type PBRs used in this study have the advantages of high mass transfer, good mixing with low shear stress and low energy consumption) (Ugwu et al., 2008). Thus, after optimizing the microalgae cultivation in indoor system, the microalgae culture was scaled up to 50 liter in vertical tubular-type PBRs. The optimal phototrophic cultivation conditions (i.e., Basal medium; initial KNO_3 concentration at 0.313 g/L) and photoheterotrophic cultivation condition (i.e., Modified Bristol's medium; pH-stat at 7.0 with acetic acid feeding) obtained from indoor systems were applied in outdoor cultivation using the 50 L tubular-type PBR (**Figs. 1 and 2**). Since there is no circulation system in the PBR, the mixing of photoheterotrophic culture was achieved by air/CO_2 aeration. The results show that the lipid contents of microalgal biomass under both cultivation conditions increased to 35-44% after nitrogen deficiency, which is similar to the results found in indoor system. However, the biomass productivity was much lower than that obtained in lab-scale culture due mainly to the slower cell growth rate. In outdoor cultivation, the biomass productivity obtained from phototrophic and photoheterotrophic conditions were only 0.07 and 0.11 g/L/d, respectively, in contrast to 0.26 and 0.20 g/L/d obtained in indoor tests. The decrease of the biomass productivity may attribute to the shorter sunlight supply period (only 10-12 hours a day) and unstable light intensity (varied from 200 to 1500 $\mu mol/m^2 \cdot s$) in outdoor system. Although the photoheterotrophic cultivation was quite successful in outdoor experiments, the pH-stat fed-batch operation is restricted by the equipment and manual feeding of acetic acid, which is quite labor-intensive. Therefore, the following outdoor studies mainly focused on the phototrophic cultivation.

3. The effect of inoculum size on cell growth and lipid production of *Chlorella vulgaris* ESP-31 in outdoor 50 liter vertical tubular-type photobioreactor

As mentioned earlier, the biomass productivity obtained from the outdoor cultivation is quite low compared to that of lab-scale tests. One of the possible ways to improve this is to use an appropriate inoculum size, which is shown to be a critical factor influencing microalgal growth, especially for outdoor cultivation systems (Rodolfi et al., 2009). Therefore, the effect of inoculum size on the growth of *C. vulgaris* ESP-31 was studied. The time-course profile of biomass production, lipid content, temperature and light intensity were illustrated in **Fig. 3**. When the inoculum size was increased from 0.25 to 0.70 g/L, the final biomass production was more than doubled (**Fig. 3**), and the biomass productivity also increased from 0.06 to 0.11 g/L/d. The lipid content increased significantly after the cell growth entered the stationary phase, and the lipid contents of low-inoculum groups were higher than that of the high-inoculum one (**Fig. 3**). Nevertheless, the highest lipid productivity (48 mg/L/d) was still obtained by using the highest inoculum (i.e., 0.70 g/L) examined. This lipid productivity is comparable with data reported in the literature. After lipid accumulation lasted for one more week, the lipid contents were all above 30% and the color of the microalgal cells clearly turned light green in all experiments.

4. Two-stage lipid accumulation strategy

Although *C. vulgaris* ESP-31 cannot maintain its growth for a long time under outdoor system, a two-

stage lipid accumulation strategy was developed to obtain microalgal biomass with a high lipid content. In the first stage, the microalga was cultivated on nitrogen-sufficient medium under outdoor system for 7 days to achieve high biomass production. After that, the microalgal cells were transferred to an indoor system to stimulate the accumulation of the lipid content. The indoor lipid accumulation tank (working volume = 8 liter) was illuminated at a light intensity of 300 μmol/m^2·s and aerated with 2% CO_2 at 0.2 vvm. The lipid contents of the microalgal biomass in the original outdoor tubular PBR was increased from 13.5% to 42.3% after cultivation for 27 days. In contrast to the outdoor tubular PBR, the lipid contents of the microalgal biomass was rapidly increased from 13.5% to 44.5% during the operation time of 23 days with combined with indoor and outdoor system. Hence, the lipid productivity increased from 43.8 mg/l/d to 54.2 mg/l/d. This may be due to the indoor tubular PBR providing uniformly light intensity (maintained at tubular PBR) and stable temperature. This also indicates that a two-stage lipid accumulation strategy is efficient in enhancing the lipid content and lipid productivity of *C. vulgaris* ESP-31.

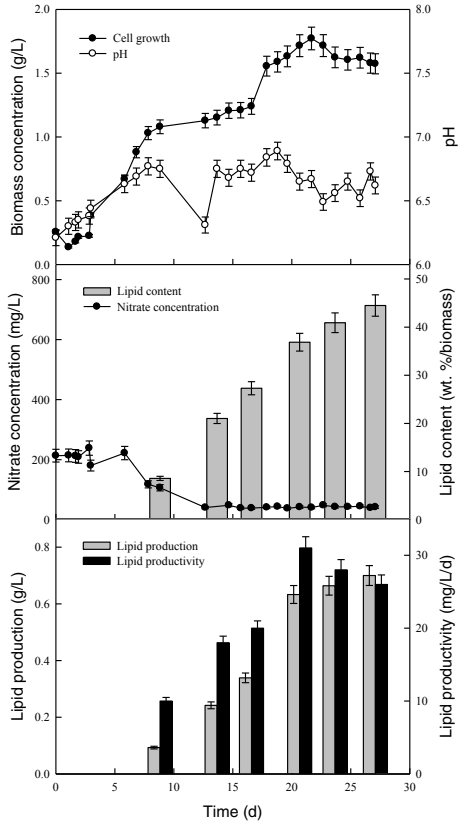

Fig.1 Time-course profiles of biomass concentration, nitrogen utilization, lipid content, lipid productivity of *Chlorella vulgaris* ESP-31 grown phototrophically in outdoor 50 L tubular PBR (medium, Basal medium; carbon source, CO_2 2.0%, 0.05 vvm)

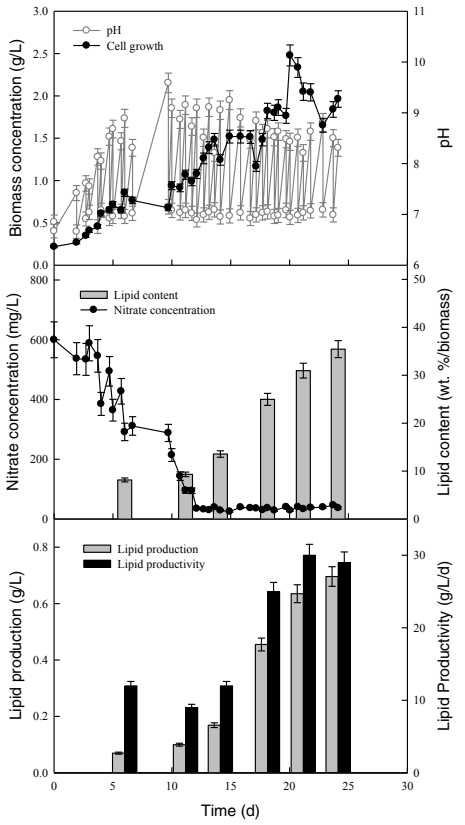

Fig.2 Time-course profiles of biomass concentration, nitrogen utilization, lipid content, lipid productivity of *Chlorella vulgaris* ESP-31 grown photoheterotrophic in outdoor 50 L tubular PBR (medium, Modified Bristol's medium; carbon source, acetate 1000 mg/L and Air 0.05 vvm)

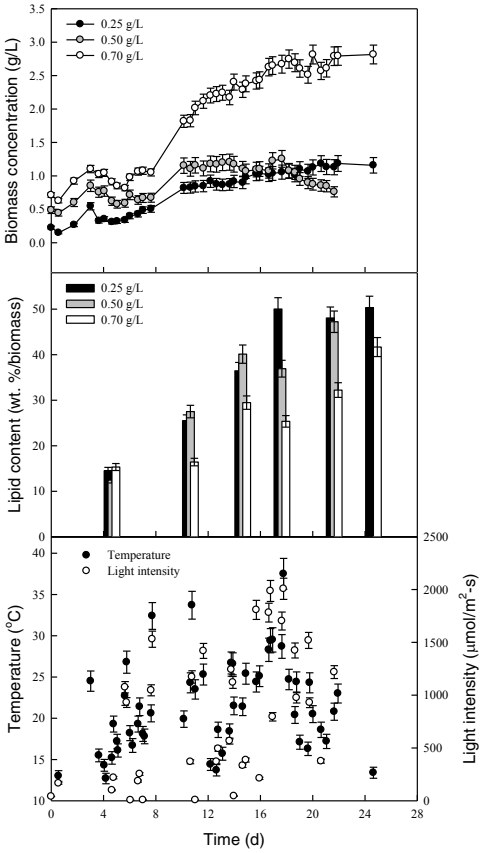

Fig.3 Time-course profiles of biomass concentration, lipid content, temperature and light intensity of *Chlorella vulgaris* ESP-31 grown phototrophically under different inoculum sizes in outdoor 50 L tubular PBR.

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