Enhancement of Lipid Production of *Chlorella pyrenoidosa* Cultivated in Municipal Wastewater by Magnetic Treatment

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Abstract. Despite the significant breakthroughs in research on microalgae as a feedstock for biodiesel, its production cost is still much higher than that of fossil diesel. One possible solution to overcome this problem is to optimize algal growth and lipid production in wastewater. The present study examines the feasibility of using magnetism treatment for algae lipid production enhancement when do outdoor cultivation of *Chlorella pyrenoidosa*. In this study, the effect of magnetic field intensity and treatment time on algal growth and lipid production was determined. Results indicated that magnetic treatment enhanced biomass and lipid production of *C. pyrenoidosa* by 14.87% and 29.79%. It was concluded that magnetism treatment is an efficient method for algae lipid production enhancement.

Introduction

Microalgae are considered as one of the most promising feedstocks for biodiesel production nowadays [1-4]. However, the main drawback for economical biodiesel production from microalgae is the high cost of algal cultivation for the huge consumption of freshwater resources, nitrogen and phosphate, and CO_2 [5-9]. One possible solution to overcome this problem is to cultivate algae in municipal wastewater due to its abundance and enrichment of nutrients [10-14]. It was found that the effluent after primary sedimentation is more conducive to the microalgal growth and accumulation of lipids compared with the effluent of secondary clarifier because of the rich nutrition [15, 16]. And Han et al. found that there was no significant differences in lipid productivity of S. obliquus grown in primary settled or sterilized municipal wastewater. Considering the high cost of sterilization and unfeasibility of large-scale application, pretreatment of municipal wastewater by sedimentation is a superior choice [17].

On the other hand, biomass and lipid production need to be enhanced which is beneficial to decrease the cost of downstream processing [18]. Among the methods, magnetic treatment has the advantages of convenient use, low running cost, non-toxic, no secondary pollution and so on. Studies showed that magnetics can lead to changes of organisms in morphology, behavior, physiology function [19]. Liu [20] and Sivasubramanian et al. [21] reported that magnetic field can promote the growth and increase the lipid content of Chlorella vulgaris and Desmococcus olivaceous, cultivated by artificial medium respectively. And Chlorella sp. [22-24] is one of the algal species which has high lipid yield and can be cultivated in wastewater.

Since few researchers tried magnetic on enhancement of algae lipid production using wastewater as the medium, the present work was intended to throw some light on feasibility of using magnetism treatment for algae lipid production enhancement and wastewater treatment when do outdoor cultivation of *C. pyrenoidosa*, providing a good way for wastewater utilization.

Materials and Methods

Algae Strain and Growth Conditions. *C. pyrenoidosa* obtained from Freshwater Algae Culture Collection at the Institute of Hydrobiology (strain number FACHB-9) was cultivated in a beaker of 2000 mL using municipal wastewater as the medium. The experiments were performed at Harbin Institute of Technology, Shenzhen, China (N 22° 35′ 25″, E113° 59′ 01″) during autumn. During the experiment, the weather was in a stable condition, and all were sunny days with maximum intensity of illumination from 70000 to 100000 lux， average temperature between 24˚C-27˚C.

Wastewater Used in the Experiment. Wastewater was collected by pumping from sewage well at the campus of Harbin Institute of Technology, Shenzhen Graduate School, Shenzhen, China. The main source of the wastewater comes from student domes and teaching building complex. After settled in a plastic bucket, the supernatant was collected for the algal cultivation, and the water quality parameters were as follows: COD 130±10 mg/L， TN 31±5 mg/L， NH_3-N 28±5 mg/L， TP 4.5±0.5 mg/L， pH 7.5±0.8.

Experimental Set-up. Experimental system diagram as shown in Figure 1, algae liquid was transferred into the magnetic device (made by Shenzhen YiTian magnetic materials co., LTD) through the creeping pump, and the algae liquid was returned to the original culture medium after the magnetic treatment. Using different magnetic intensity and magnetic cycle times to treat algae solution. The effect of different magnetic treatment intensity and different magnetic cycle times on the growth and lipid production of *Chlorella pyrenoidosa* cultured in municipal wastewater was studied. Finally the optimum magnetic treatment conditions was determined.

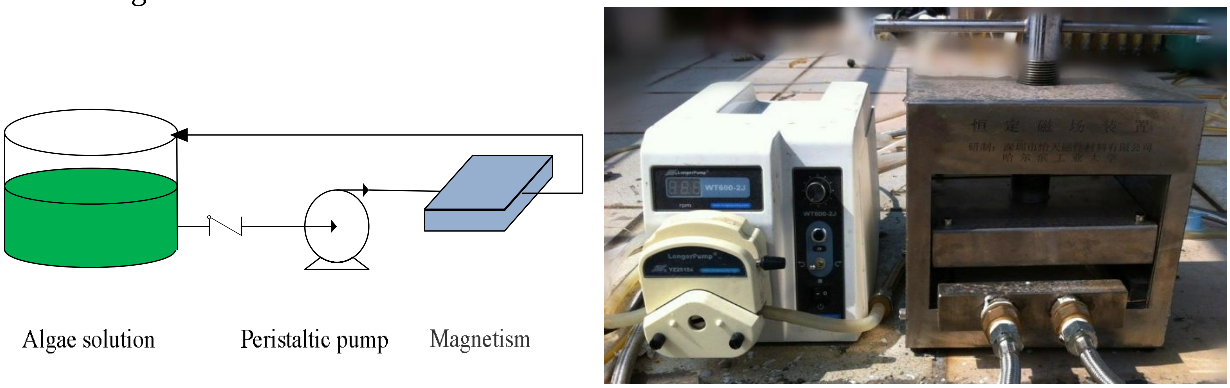


Figure 1 Schematic diagram of the process for magnetism treatment

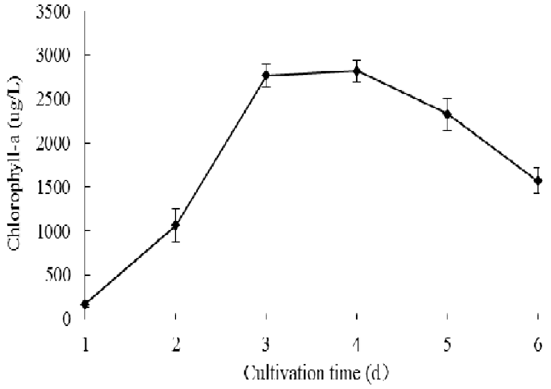
Biomass Assay. Algal growth was monitored by the measurement of Chlorophyll-a using an YSI fluorescent chlorophyll meter (YSI 600CHL, USA) and by determination of algal cellular dry weight (CDW). Using the values of Chlorophyll-a, a linear correlation was obtained following the logarithmic growth model, and the values of the specific growth rate (ug L-1 day-1) were calculated from the corresponding slope. When measured CDW, take a certain amount of algae fluid to centrifuge for 10 min under 8000 r/min, discard the supernatant, and transfer the algae with distilled water in pre-weighed weighing bottle, then dried in oven at 105 ˚C until the weight was constant.

Lipid Analysis. Extraction of lipids was done by the modified Folch method [25]. The lipid extracts were dried under a stream of argon. The pre-weighted glass vials containing the lipid extracts were dried at 80 °C for 30 min, cooled in a desiccator and weighted.

Data Analysis. All of the values were the means of three replicates. Microsoft Excel 2010 was used for data treatment and standard deviation analysis. Data obtained were analyzed statistically to determine the degree of significance at $P \leq 0.05$ using one-way analysis of variance (ANOVA) with SAS version 6.12.

Results and Discussion

The growth and lipid production of *C. pyrenoidosa* without magnetic treatment. *C. pyrenoidosa* got into stationary growth stage in 3 days and after 4 days of cultivation, went into the decline phase because of the nutrients consumption in wastewater without magnetic treatment (Fig. 2). According to the growth of *C. pyrenoidosa* in the municipal wastewater, the biomass and lipid production were measured (Fig. 3). The biomass and lipid production reached the highest value after 4 days, respectively, 0.45 g/L and 0.20 g/L. Therefore, *C. pyrenoidosa* cultivated for 4 days was harvested for further experiments.



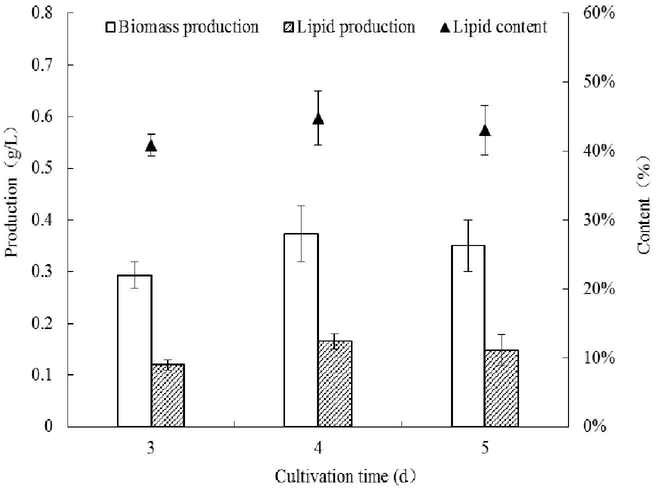
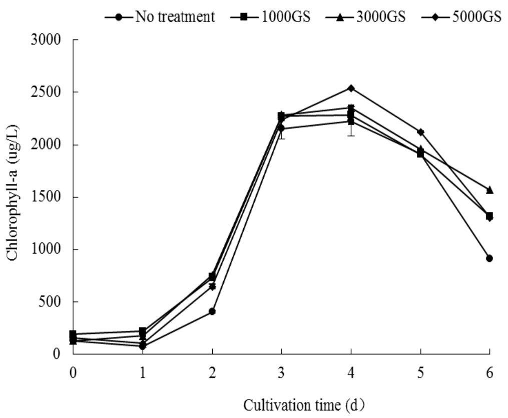


Figure 2 The growth of *C. pyrenoidosa* without magnetic treatment
Figure 3 The biomass and lipid production of *C. pyrenoidosa* without magnetic treatment

Effects of magnetic field intensity on the growth and lipid production of *C. pyrenoidosa*. The effect of magnetic field intensity on the growth and lipid production of *C. pyrenoidosa* were measured in three different magnetic field intensity (Fig. 4 and Fig. 5). When the magnetic field intensity increased from 1000GS to 5000 GS, the concentration of chlorophyll-a increased. The highest concentration of chlorophyll-a was obtained using 5000 GS, 14.29 % higher than the corresponding control. Figure 5 showed different magnetic field intensity also increased the biomass and lipid production. The highest biomass and lipid production were obtained using 5000 GS, 38.00 % and 57.69% higher than the corresponding control. Therefore, magnetic field intensity of 5000 GS was used for further experiments.



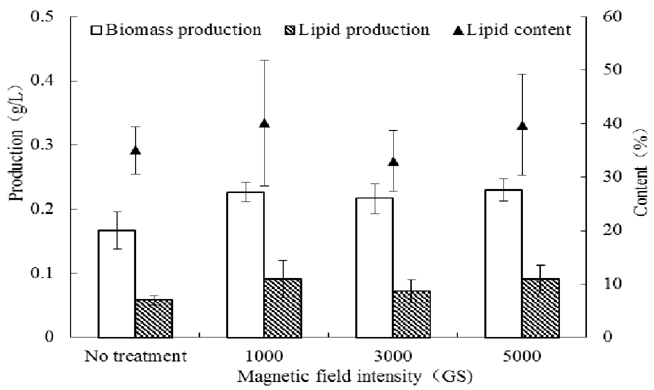
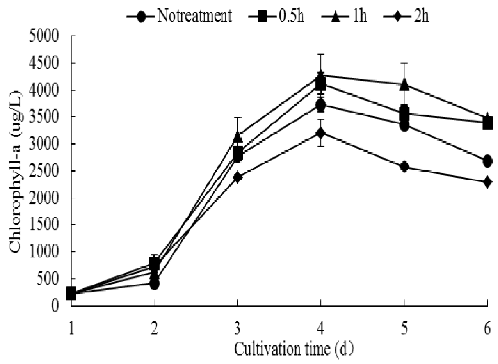


Figure 4 The effect of magnetic field intensity on Chlorophyll-a of *C. pyrenoidosa*

Figure 5 The effect of magnetic field intensity on the biomass and lipid production of *C. pyrenoidosa*

Effects of magnetic treatment time on the growth and lipid production of *C. pyrenoidosa*. Increasing treatment time can extend the exposure time of cells to magnetic field, thereby enhancing the treatment effect. Exposure of cells to magnetic field for different time (0.5, 1 hours) resulted in increase the concentration of *chlorophyll-a* by 10.25, and 14.58%, respectively, with respect to the control (Fig. 6). However, 2 hours treatment showed a decrease in the concentration of *chlorophyll-a* to the control. Using different treatment time also showed significant increase in biomass and lipid production by increasing treatment time from 0.5 h to 1 h, and then showed a decrease at 2 h (Fig. 7). Treatment of *C. pyrenoidosa* cells with 2 h resulted in 14.87 % increase in lipid yield with respect to the control. In addition, 2 h showed 29.79% significant increase in lipid yield with respect to the untreated cells. The results showed that there was a significant difference in lipid production of *C. pyrenoidosa* cultivated with and without magnetic treatment. This confirmed the feasibility of using magnetic field to enhance lipid production of *C. pyrenoidosa* using wastewater as medium. Therefore, treatment of cells with 5000 GS for 2 hours was nominated as optimum conditions.



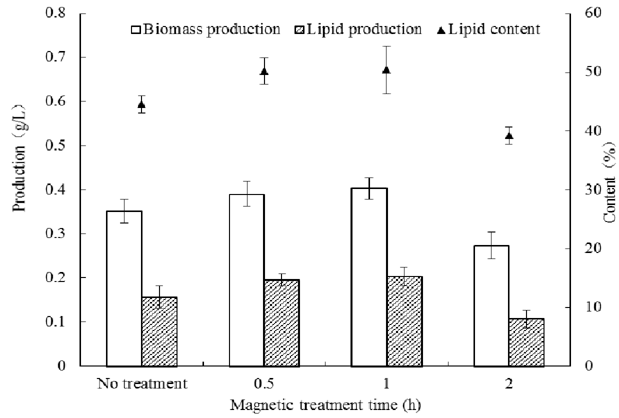


Figure 6 The effect of magnetic treatment time on Chlorophyll-a of *C. pyrenoidosa*

Figure 7 The effect of magnetic treatment time on the biomass and lipid production of *C. pyrenoidosa*

Sivasubramanian et al. [21] reported that pulsed magnetic field can promote the growth and increase the lipid content of *Desmococcus olivaceous* by 40% and 16% respectively. According to Liu, magnetic field can both promote and inhibit the growth of microalgae, and usually the magnetic field intensity for improving algae growth is low [20]. Takahaski and Toshiyuki [26] found that the specific growth rate of *Chlorella vulgaris* almost doubled using 400 Gs magnetic field application. While in our work, when using 5000Gs magnetic treatment to cultivate *C. pyrenoidosa*, it also grew well. This means that *C. pyrenoidosa* has a strong adaptability to high-intensity magnetic field which is in consistent with Liu who found that after magnetic treatment with 1800Gs, 16 of the 17 species died, except *Chlorella* increased from 13.7 cell/mL to 73800 cell/mL [20].

The results showed the feasibility of using magnetism treatment for continuous algae lipid production and wastewater treatment when do outdoor cultivation of *C. pyrenoidosa,* providing a good way for wastewater utilization.

Conclusions

The results from this study demonstrated the feasibility of using magnetism treatment for algae lipid production enhancement when do outdoor cultivation of *C. pyrenoidosa*. Research is continuing in our department to identify the feasibility of *C. pyrenoidosa* lipid as a feedstock for biodiesel in long run outdoor cultivation. Results indicated that magnetic treatment enhanced biomass and lipid production of *C. pyrenoidosa* by 14.87% and 29.79% with 5000 GS for 2 hours was nominated as optimum conditions. It was concluded that magnetism treatment is an efficient method for algae lipid production enhancement.

Acknowledgements

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