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## Effects of light and pH on cell density of *Chlorella vulgaris*

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### Abstract

Improving the cell density of microalgae cultivation is one of the keys to reduce the cost of microalgae biodiesel. Many studies showed that the adjustment of pH and light intensity could increase cell density. The effects of light intensities, pH and pH adjustments on the growth of *Chlorella vulgaris* were studied in light incubator. The light intensities were set at 3960, 7920 and 11920lux; values of pH were 7, 8, 9 and 10 respectively; and pH adjustment methods included without and with pH control. Results show that: (1) In terms of light intensity, without pH control, the cell density under 3960lux is highest. With pH control, the cell density under 7920lux is higher than other levels. (2) In terms of pH, under the same light intensity, the cell density with pH control at 10 is highest, which indicates the light intensity will not affect the optimal pH value. And the pH fluctuates between 10 and 10.5 with pH control at 10, which is the most suitable range of pH for *Chlorella vulgaris* cultivation. (3) For pH adjustment methods, under 7920lux, the cell density with pH control at 10.0 is 56.7% higher than that with initial pH at 10.0, while the cell density with initial pH at 7.0 is 34.7% than that with pH control at 7.0, which indicates the method with pH control at values of the optimum pH makes better growth of microalgae.

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**Keywords:** *Chlorella vulgaris*; light intensity; pH; cell density

### 1. Introduction

Microalgae are regarded as the most likely raw material to replace petroleum, for high oil levels, short life cycles, and carbon sequestration [1]. However, low cell density of microalgae cultivation system is one of bottle-necks for commercial production, which leads to high costs of microalgae biofuels [2]. Thus, improving the cell density has great significance for industrialization of microalgae biodiesel.

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Studies show that light and pH are important factors which affect the growth of autotrophic microalgae. Kitaya Y et al. [3] showed the growth rate of *Euglena gracilis* first increased then decreased with increase of light intensity ( $20\text{-}200\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Khalil ZI et al. [4] showed *Chlorella* can grow in a wide pH range (4-10) and the maximum biomass yield was obtained in alkaline conditions (pH at 9 and 10).

Thus, light intensity and pH are very important for microalgae cultivation. In this paper, the effects of light intensities, pH and pH adjustments on *Chlorella vulgaris* were studied to gain high cell density.

## 2. Materials and methods

*Chlorella vulgaris* (FACHB-1227) were bought from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB, China). *Chlorella vulgaris* were cultured 20 days, with 250mL BG11 medium in 500mL conical flasks in light incubator. The inoculum algae density was  $10^6$  cells/mL.

The light intensities were set at 3960, 7920 and 11920lux. The light/dark period was 12h/12h. Moreover, the temperature in the light period was  $25\pm 1^\circ\text{C}$ , and  $20\pm 1^\circ\text{C}$  in the dark period to reduce the cellular respiration. The pH was set at 7, 8, 9 and 10 respectively; and pH adjustment methods included with and without pH control. The method of with pH control refers to daily adjusting the pH using NaOH or HCl solutions, while the method of without pH control refers to only adjusting the value of pH at the beginning of cultivation. Moreover, the experiments with pH control were carried out in duplicate.

The cell density ( $X$ ) after 20 days cultivation was determined by the optical density at 686 nm ( $OD_{686}$ ) of cells with a spectrophotometer [5]. And the relation is shown as Equation (1).

$$X = 11.57 \cdot OD_{686} - 0.38 \quad (R^2 = 0.9863) \quad (1)$$

## 3. Results and discussion

### 3.1. Effect of light intensity on the cell density of *Chlorella vulgaris*

Seen from Fig.1, without pH control, the cell density under the light intensity of 3960lux is highest in various pH levels, while the least was obtained under 11920lux. With initial pH at 7.0, cells under 3960lux grew fast, up to  $14.62263 \times 10^6$  cells/mL, which was 23% and 45.4% higher than that under 7920 and 11920lux respectively. Moreover, the pH under 3960lux rose from 7.0 to 10.0. While the pH under 7920 and 11920lux increased and remained stable around 10.8, which may be the main reason for lower cell density. The results with initial pH at 8.0, 9.0 and 10.0 were similar with that obtained with pH at 7.0. Yeh K-L et al. [6] observed a high final pH of 11 and the high pH inhibited the *Chlorella vulgaris* growth.

With pH control, the cell density under 7920lux is highest in various pH levels, while the least was obtained under 11920lux. With pH control at 7.0, the cell density under 3960lux was less than that under 7920lux in the early culture period, but caught up during the end of cultivation, which was 1.58 times than the cell density under 11920lux. Moreover, the pH under 3960 and 11920lux fluctuated from 7.0 to 8.5, while the pH under 7920lux fluctuated from 7.0 to 9.0. With pH control at 8.0 and 9.0, cell density under 7920lux was highest. Moreover, with pH control at 10.0, the cell densities under 3960 and 7920lux were almost same, 29.7% higher than that under 11920lux.

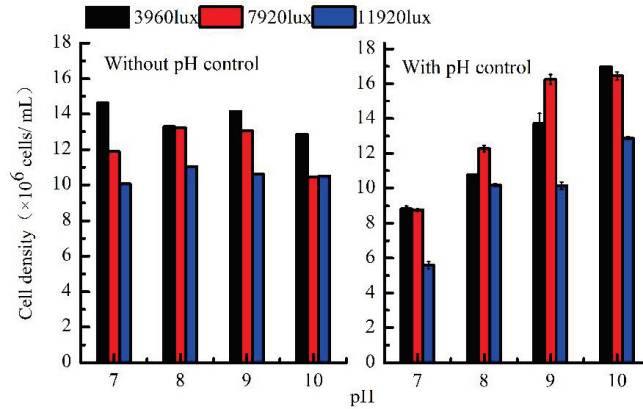


Fig.1 Effects of light intensity on the cell density with and without pH control

It can be suggested in terms of light intensity, with initial pH adjustment, the cell density under the light intensity of 3960lux is highest in various pH levels. With pH control, the cell density under 7920lux is higher than that under other light intensities in various pH levels.

### 3.2. Effect of pH on the cell density of *Chlorella vulgaris*

Shown in Fig.2, under the same light intensity, the cell densities had very small differences in various initial pH levels. Çelekli A and Dönmez G [7] showed that with initial pH at 6.0, 7.0 and 8.0, the cell densities of *Dunaliella sp.* were about  $(3.95 \pm 0.15) \times 10^6$  cells/mL. And it also suggested that the initial pH levels have little impact on cell density.

With pH control, the cell density under the three light intensities all increased with the increase of pH, and was highest with pH control at 10.0. Moreover, the pH of culture broth with pH control at 10.0 fluctuated between 10.0 and 10.5. That's mainly because the pH control refers to daily adjusting the pH, which limits the rising of pH. It can be suggested that the light intensity will not affect the optimal pH value. Meanwhile, the pH of culture broth with pH control at 10.0 fluctuated between 10.0 and 10.5, which was the optimal range for the growth of *Chlorella vulgaris*.

### 3.3. Daily variation of cell density with and without pH control

Under 3960lux, the cell density with pH control at 10.0 was  $16.956 \times 10^6$  cells/mL, 1.32 times than that with initial pH at 10.0. While, in terms of pH at 7.0, 8.0 and 9.0, the cell densities with pH control were less than that without pH control. This was mainly because without pH control, the pH quickly rose and remained stable around 10.0, which was good for cell proliferation; and the pH with pH control was far away from the optimum range, resulting in lower cell density. It can be suggested that the pH adjustment method which keeps microalgae stay longer in optimum pH range, makes better growth of microalgae.

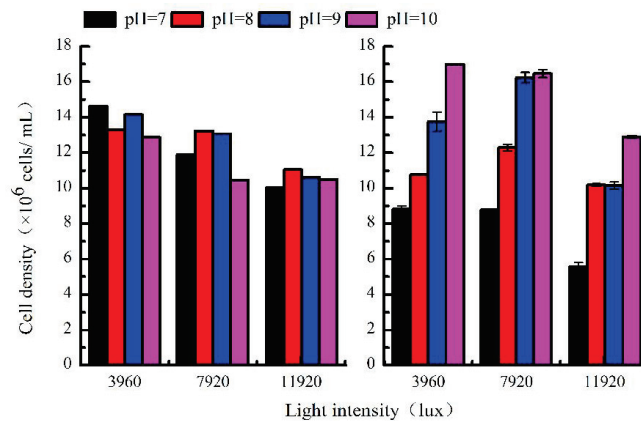


Fig.2 Effects of pH on the cell density under three light intensities

#### 4. Conclusion

Here the effects of light intensities, pH and pH adjustments on the growth of *Chlorella vulgaris* were studied in light incubator and we concluded that: (1) under the light intensity of 7920lux, the cell density with pH control at 10.0 is highest than other groups. (2) The most suitable range of pH for *Chlorella vulgaris* cultivation is between 10 and 10.5, and light intensity will not affect the optimal pH value. (3) The method with pH control at values of the optimum pH makes better growth of microalgae.

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