Journal of Photochemistry & Photobiology, B: Biology 162 (2016) 619–624

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Adjusting irradiance to enhance growth and lipid production of *Chlorella vulgaris* cultivated with monosodium glutamate wastewater

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ARTICLE INFO

Article history:
Received 14 March 2016
Received in revised form 3 July 2016
Accepted 21 July 2016
Available online 26 July 2016

Keywords:
Monosodium glutamate wastewater
Chlorella vulgaris
Light intensity
Biochemical composition
Specific growth rate

ABSTRACT

Light is one of the most important factors affecting microalgae growth and biochemical composition. The influence of illumination on *Chlorella vulgaris* cultivated with diluted monosodium glutamate wastewater (MSGW) was investigated. Six progressive illumination intensities (0, 30, 90, 150, 200 and 300 $\mu mol \cdot m^{-2} s^{-1}$), were used for *C. vulgaris* cultivation at 25 °C. Under 150 $\mu mol \cdot m^{-2} s^{-1}$, the corresponding specific light intensity of 750×10^{-6} $\mu mol \cdot m^{-2} s^{-1}$ per cell, algae obtained the maximum biomass concentration (1.46 $g \cdot L^{-1}$) on the 7th day, which was 3.5 times of that under 0 $\mu mol \cdot m^{-2} s^{-1}$, and the greatest average specific growth rate (0.79 d^{-1}) in the first 7 days. The results showed the importance role of light in mixotrophic growth of *C. vulgaris*. High light intensities of 200 and 300 $\mu mol \cdot m^{-2} s^{-1}$ would inhibit microalgae growth to a certain degree. The algal lipid content was the greatest (30.5%) at 150 $\mu mol \cdot m^{-2} s^{-1}$ light intensity, which was 2.42 times as high as that cultured in dark. The protein content of *C. vulgaris* decreased at high light intensities of 200 and 300 $\mu mol \cdot m^{-2} s^{-1}$. The effect of irradiance on carbohydrate content was inversely correlated with that on protein. The available light at an appropriate intensity, not higher than 200 $\mu mol \cdot m^{-2} s^{-1}$, was feasible for economical cultivation of *C. vulgaris* in MSGW.

1. Introduction

Microalgae cultivation has attracted extensive attention around the world because of its advantages of rapid growth, CO_2 fixation, good tolerance to environmental factors, and accumulation of nutrient (e.g. protein, lipid, carbohydrate) [1–3]. However, mass culture of microalgae for obtaining value-added products has not been commercialized, mainly due to high cost and low productivity. According to Kovacevic and Wesseler [4] the cost of biomass production accounts for 43.7% of total input for algal biodiesel utilization, while carbon and water account for about 35% of the microalgae biomass production. Thus, utilization of available and low-cost water supply is crucially important for the success production of microalgae biofuel [5]. In order to reduce the cost, many researchers have explored the cultivation of microalgae with various kinds of wastewater with abundant N and P. Wu et al. [6] reported *Chlamydomonas* sp. cultivated with industrial wastewater could accumulate lipid up to 18.4% and more than 90% of total fatty acids are suitable for biodiesel production. Abreu et al. [7] studied the mixotrophic cultivation of *Chlorella vulgaris* in hydrolyzed cheese whey and found the productivities of algal biomass, protein, lipid and starch are much higher than those in the photoautotrophic control culture. The strategy of cultivating microalgae with wastewater would minimize the demand for freshwater and decontaminate wastewater, as well as reducing the cost of biomass production.

Monosodium glutamate wastewater (MSGW) containing abundant nutrient is ideal alternative for *Chlorella vulgaris* cultivation as shown by previous research [8]. Different nutrients concentration achieved by changing the diluting time of MSGW have been researched, finding that the highest biomass and lipid productivity of microalgae were obtained in 100-time diluted MSGW, which were about treble of that in BG11 medium. Besides the cultivation medium, the growth of microalgae would also be affected by a variety of environment factors, among which light is an important element [9–11]. When the light supply is insufficient, the growth of microalgae is improved by the enhancement of irradiance up to the photo-saturation, while higher light intensity exceeding the photo-saturation point will inhibit algal growth [12]. Moreover the diluted MSGW is brown which influences the light absorption of algae, adjusting irradiance might promote growth and biochemical compositions accumulation of *C. vulgaris* in this broth significantly.

During biodiesel production, microalgae growth stage consumes most of the energy, mainly the power supply for equipment [13]. Thus, appropriate illumination condition will not only increase

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http://dx.doi.org/10.1016/j.jphotobiol.2016.07.025
1011-1344/

microalgae productivity but also avoid energy waste. The objective of this paper was to investigate the influence of different light intensities on the biomass concentration and specific growth rate of *Chlorella vulgaris* cultivated in 100-fold diluted MSGW. Moreover, the biochemical composition in different light intensities was analyzed. The optimal light intensity was determined for algae biomass and biocompounds production with little consumption of light energy.

2. Methods and Materials

2.1. Microalgae and Media

Chlorella vulgaris supplied by Culture Collection of the Institute of Hydrobiology (FACHB-Collection, China) was used in this study. It was preserved and activated in BG11 medium as Ji et al. [8] described.

The monosodium glutamate industrial wastewater (MSGW) was obtained from a monosodium glutamate plant in Chiping County, Shandong Province. The detailed characteristics of this wastewater are as follows: pH of 2.5 ± 0.1, COD of 500 ± 4 g·L^{-1}, BOD_5 of 160 ± 2 g·L^{-1}, TN of 7.0 ± 0.2 g·L^{-1}, NH_3-N of 1.0 ± 0.1 g·L^{-1}, TP of 2.41 ± 0.11 g·L^{-1}. Prior to experiment the MSGW was diluted 100 times with purified water and sterilized at 120 °C for 30 min. Before sterilization the pH of this broth was adjusted to 7.5 using 1.0 mol·L^{-1} NaOH solution.

2.2. Experimental Setup

The *C. vulgaris* inoculated into BG11 medium was cultivated at 25 °C and continuous illumination of 30 μmol·m^{-2} s^{-1}. When the microalgae growth was at logarithmic phase, it was used as seed after being centrifuged at 4000 rpm for 10 min and washed with 0.5 mol·L^{-1} $NaCO_3$ solution. The bath experiments were carried out in Erlenmeyer flasks (1 L), which were incubated at 25 °C in illumination incubators under continuous illuminations of 0, 30, 90, 150, 200 and 300 μmol·m^{-2} s^{-1} and shook six times every 3 h from 7:30 to 22:30 every day. The light intensity on surface of the flasks was measured using an irradiance sensor (ZDS-10, Shanghai Cany Precision Instrument, China). The reactors containing about 800 mL prepared MSGW medium were added with a sample tap on one side and sealed with 8-layer gauzes. A control experiment was carried out simultaneously in BG11 medium. To avoid contamination from other microorganism, the antibiotic of Levofloxacin Hydrochloride Tablets was added to every reactor with its final concentration of 20 mg·L^{-1}. An amount of seed was inoculated into each reactor to meet the final cell concentration of 0.2 × 10^6 cells·mL^{-1}. Each experiment with different light intensities was made in triplicate to verify the reproducibility of the data obtained in this work.

2.3. Growth Measurement of Chlorella vulgaris

The evaluation of algal growth was carried out through everyday biomass concentration (g·L^{-1}, dry weight (DW)) and then calculated the specific growth rate (μ, d^{-1}) as Ji et al. [8] did.

The electricity conversion efficiency of light was calculated followed the Eq. (1) and hypothesis of dark condition in the report of Li et al. [14].

$$\text{Electricity conversion efficiency} = DW/\text{light intensity} \quad (1)$$

for dark condition, the light intensity was thought as 1 μmol·m^{-2} s^{-1} to calculate the electricity conversion efficiency.

2.4. Analysis of Biomass Components

Microalgae biomass was collected by centrifugation, dried to constant weight after cultivation and analyzed the biomass components according to the previous report [8]: (1) amino acids of algae biomass were analyzed for quality and quantity using an amino acid analyzer (L-8900, Hitachi, Japan) and the essential amino acid scores (EAAS), chemical scores (CS) and essential amino acid index (EAAI) were calculated, to assess the influence of light intensity on protein nutritional quality; (2) the protein content of the microalgae biomass was represented by the content of total amino acid; (3) total lipid of microalgae was extracted and quantified gravimetrically and fatty acid profiles were analyzed by GC-MS; (4) after hydrolysis of microalgae powder, the content of total carbohydrate was determined.

The average productivities (mg·L^{-1} d^{-1}) of protein, lipid and carbohydrate were calculated according to the following formula:

$$P = (DW \times \omega)/T \quad (2)$$

where DW is the biomass concentration (mg·L^{-1}) on the day of harvest, ω represents the weight percent of each biochemical component (%), including protein, lipid and carbohydrate, and T is the incubation time (d).

2.5. Nutrients Removal in the MSGW Media

The concentrations of total nitrogen, total phosphorus and chemical oxygen demand (COD) in each medium were measured after harvest of microalgae according to standard methods [15]. The tests on each index were carried out in triplicate.

3. Results and Discussion

3.1. Microalgae Growth

The growth of microalgae, as indicated by dry weight and specific growth rate under different light intensities, is shown in Fig. 1. The growth of algal biomass was affected significantly by the variation of

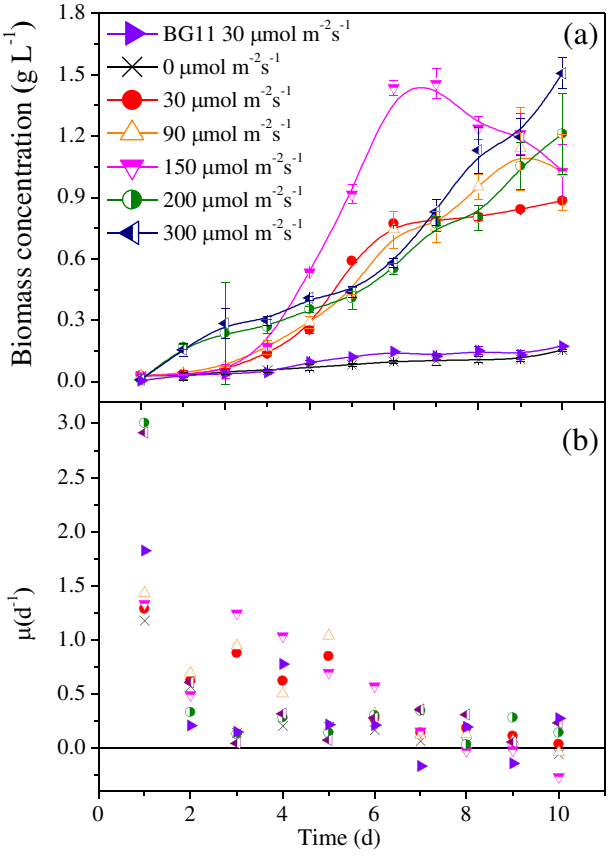

Fig. 1. Temporal variation of the (a) biomass concentration and (b) specific growth rate for different light intensities.