Different plastic-bag type photobioreactor for biomass production of *Chlorella* species

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Abstract. Microalgae were generally recognized as safe and a promising source for proteins, polyunsaturated fatty acids, carbohydrates, and other bioactive substances in the industrial application such as foods, health supplements, biofuels, and pharmaceuticals. For mass production and cost down, plastic-bag has been utilized for microalgal growth. In this study, three plastic-bag types (plastic-bag, vertical plastic-bag, and horizontal plastic-bag) were designed to shape the liquid circulation for improving the mass transfer. Microalgae were cultivated under different weathers including the sunshine times and average global radiation as 46.7 h and 9.15 MJ/m², 13.7 h and 4.8 MJ/m², 38.2 h and 8.07 MJ/m², and 37.1 h and 11.25 MJ/m² for 15 days. The pH, biomass, and antioxidant of microalgae were further detected. The pH values of microalgae were ranged from 7.3–9.0 within 15 days cultivation. No obvious difference was present among these plastic-bags. Under low global radiation with the average 4.8 MJ/m², the microalgal biomass produced by the vertical plastic-bag was 1.4-fold and 1.9-fold higher than that of the plastic-bag and horizontal plastic-bag, respectively. This result suggested that the vertical plastic-bag photobioreactor had a high mass transfer with low shear stress. Furthermore, the antioxidant activity was assayed by the total polyphenol and total reductive capability. The result revealed that the microalgae contained 360.4 µg/L-culture corresponding to gallic acid using vertical plastic-bag, followed by horizontal plastic-bag with 289.6 µg/L-culture and plastic-bag with 230.6 µg/L-culture by the total polyphenol determination after 15 days cultivation. The similar result was also observed in the total reductive capability by vertical plastic-bag with 413.4 µg/L-culture corresponding to ascorbic acid, followed by horizontal plastic-bag with 373.2 µg/L-culture and plastic-bag with 353.7 µg/L-culture. Consequently, the vertical plastic-bag had the potential to be applied in the industrial field.

1. Introduction

Comparing with the traditional agriculture for oil crop, microalgae grew rapidly and required a low area for cultivation [1]. They can cultivate in pollutant environment or non-agricultural land, and do
not compete with other crops for food production. Microalgae accumulate large amounts of proteins, oils, and carbohydrates which can be used for healthy food, bioenergy, and pharmaceuticals [2]. Several essential amino acids are abundant in microalgae. Additionally, various carbohydrates such as starch, glucose, cellulose, hemicellulose, xylose, galactose, rhamnose, arabinose, and fucose were found, which can be applied in alcohol fermentation [3]. Microalgae usually contain essential fatty acids such as linolenic acid (C18:2), alpha-linolenic acid (C18:3) and arachidonic acid (C20:4) which provide an alternative to vegetable oils [4]. Moreover, ω-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6) offered good effects on the brain, retinal development, and cardiovascular health are present in some algae. On the other hand, high-value products as β-carotenoid and astaxanthin revealing high antioxidant activity also can be obtained through microalgae cultivation. Thus, some species such as Chlorella vulgaris, Dunaliella salina, Haematococcus pluvialis, Spirulina maxima, Nannochloropsis and Tetraselmis has been commercialized for nutritional supplements and animal feeds [5-6].

Microalgae culture can be divided into an open pond and a closed photobioreactor [7]. Although the open pond system including a circular pond and a raceway pond can be mass-produced and cost-effective, climate and pollution problems are the disadvantages of this technology. Closed photobioreactor contains flat-plate, tubular and column. The advantages of closed photobioreactor are high yield, controllable, low CO₂ loss, high culture density and not easily contaminated; however, high equipment cost is necessary [7]. Plastic-bags combine the advantages of these two technologies with low cost and less contamination. In this study, three plastic-bags were designed for Chlorella sp. outdoor cultivation. The pH and biomass of Chlorella sp. production were determined during different sunshine and global radiation. The antioxidant properties of Chlorella sp. were further measured.

2. Materials and methods

2.1. Cultivation of Chlorella sp.
The medium (1.7 g urea, 0.8 g MgSO₄·7H₂O, 0.4 g KH₂PO₄, 0.005 g FeSO₄·7H₂O, 0.007 EDTA per liter containing 1 mL trace metal solution with 2.86 g H₃BO₃, 1.81 g MnCl₂, 0.22 g ZnSO₄, 0.08 g CuSO₄, 0.021 g NaMoO₄, and 10 µl H₂SO₄ per liter) was used for outdoor cultivation of Chlorella species.

2.2. Growth and biomass of Chlorella sp.
Plastic-bag was composed of polypropylene material. Three plastic-bags were designed by heat sealing machine for vertical and horizontal liquid circulation via gas introduction with 1.5 L/min. Two-liter medium was added into the plastic-bags for outdoor cultivation of Chlorella species. The microalgal growth was determined by a spectrometer at 682 nm. Biomass productivity (g/L) was calculated according a standard curve (OD value = 6.1354 × biomass (g/L) – 0.0233) [8].

2.3. Determination of total polyphenols
The total polyphenols assay was determined based on the Folin-Ciocalteu method. The Chlorella sp. was harvested, centrifuged at 10,000 xg for 10 min, and dried by freeze dryer. The microalgal sample was ground, extracted, and mixed with Folin-Ciocalteu reagent for 5 min reaction. Then, a 10% sodium carbonate solution with 0.5 mL was added to the mixture and left for 30 min in the dark. The sample was centrifuged at 10,000 xg for 10 min at room temperature. The supernatant was detected by a spectrometer at 760 nm. Various concentration of gallic acid (12.5, 25, and 50 µg/mL) was used as a positive control to calculate a standard curve.

2.4. Determination of total reductive capability
The total reductive activity was determined based on the method of Oyaizu [9]. The dried Chlorella sp. sample was added and mixed with 0.2 M sodium phosphate buffer (pH 6.6) and 1 % K₃FeCN₆ at 50°C for 20 min. After incubation, 10 % trichloroacetic acid was added, and the mixture was centrifuged at
10,000 xg for 10 min. The supernatant was mixed with H₂O and 0.1% FeCl₃. The absorbance was determined by a spectrometer at 700 nm. Various concentration of ascorbic acid (12.5, 25, and 50 μg/mL) was used as a positive control to calculate a standard curve.

3. Results and discussion

3.1. Design of plastic-bag for Chlorella sp. cultivation

The hydrodynamic and mass transfer of photobioreactors were important for microalgal culture [7, 10]. A few studies on mass transfer were reported in phototrophic cultures of microalgae [7]. Because plastic-bag was relatively economical and good for microalgal cultivation, three plastic-bag types were designed to investigate the effect of mass transfer and gas holdup on microalgal productivity (Fig. 1). The heat sealing was used to shape the liquid circulation in plastic-bag; thus, the vertical and horizontal paths of plastic-bag were created.

![Figure 1](image_url)

*Figure 1.* Three plastic-bags were designed to determine the effect of mass transfer on microalgal productivity. (a) Plastic-bag, (b) vertical plastic-bag, and (c) horizontal plastic-bag were produced by heat sealing machine.

When the air passed through the culture medium, the mass transfer from the gas to the liquid was conducted. Therefore, the bubble flow was simulated by the software of COMSOL Multiphysics (www.comsol.com). The volume fraction of bubbles was evaluated using the model of Bubbly Flow with a 2D axisymmetric component. The parameters of COMSOL Multiphysics were set up as follows: bubble diameter, 0.004 m; gas volume, 2.5E-5 m³/s; inlet velocity, 1.9894 m/s; Slip-velocity proportionality constant rhog in 0.9727[kg/(m³)]. Observing plots between t = 0.3 s and t = 1.2 s (Fig. 2), the gas bubbles were rising up and imparted momentum to the culture medium, recirculating and
forming the vortex in the plastic-bag. Comparing the convection and diffusion of the gas bubbles, the predominance of convective transport was obvious in the vertical plastic-bag.

Figure 2. Time evolution of gas bubble volume fraction in the (a) plastic-bag, (b) vertical plastic-bag, and (c) horizontal plastic-bag at t = 0.3 s, t = 0.8 s, and t = 1.2 s. The right columns indicate the volume fraction of bubbles in the plastic-bag.

3.2. Outdoor cultivation of Chlorella sp. with different sunshine times and global radiation

Figure 3. Microalgae were cultured outdoors with different sunshine times and global radiation. The sum of sunshine times and an average of global radiation were a, 46.7 h and 9.15 MJ/m²; b, 13.7 h and 4.8 MJ/m²; c, 38.2 h and 8.07 MJ/m², and d, 37.1 h and 11.25 MJ/m².
Artificial light can be applied in the microalgal culture system [11]; however, it could increase the cost of microalgal production. Comparing with the open pond, the plastic-bag had a good light path, which was similar to flat-plate photobioreactor with a large illumination surface area [12]. The light to biomass efficiency was an important factor for industrial productivity [13]. In this study, to detect microalgal production by using different plastic-bags, the microalgal culture was set up outdoors during different sunshine times and global radiation within 15 days. Four kinds of weathers are shown in Fig. 3.

Figure 4. The pH value of microalgal culture within 15 days. The pH value was determined according to the weather of (a) 46.7 h and 9.15 MJ/m$^2$, (b) 13.7 h and 4.8 MJ/m$^2$, (c) 38.2 h and 8.07 MJ/m$^2$, and (d) 37.1 h and 11.25 MJ/m$^2$.

3.3. Determination of pH and biomass of Chlorella sp. cultivation

The pH value was also difficult to keep constant growth factor [13]. Therefore, the pH was monitored during microalgal growth (Fig. 4). The pH values were ranged from 7.3~9.0 within 15 days of growth. The pH change on vertical and horizontal plastic-bag photobioreactors was not obvious under different weathers. Nevertheless, the pH of horizontal plastic-bag was slightly higher than the others. In addition, the biomass of microalgae was determined (Fig. 5). The result revealed that the vertical plastic-bag had higher biomass than the other plastic-bag types in the various weathers. Under the high global radiation, no obvious difference was observed in all plastic-bags. However, the microalgal biomass produced by the vertical plastic-bag was 1.4-fold and 1.9-fold higher than that of the plastic-bag and horizontal plastic-bag, respectively, under the low global radiation of 4.8 MJ/m$^2$. This result suggested that the vertical plastic-bag can exert a great ability of mass transfer under poor global radiation. This design was similar to the vertical column photobioreactor which had a high mass transfer with low shear stress [7, 14]. When the weather is poor outdoors, the vertical plastic-bag can be effectively used.
Figure 5. The biomass of microalgal culture within 15 days. The biomass was harvested according to the weather of (a) 46.7 h and 9.15 MJ/m², (b) 13.7 h and 4.8 MJ/m², (c) 38.2 h and 8.07 MJ/m², and (d) 37.1 h and 11.25 MJ/m².

3.4. Determination of antioxidant activity of Chlorella sp. cultivation

To comprehend the bioactive substance of microalgae, the antioxidant activity was determined by the total polyphenol and total reductive capability (Table 1). Polyphenolic compounds are recognized as the factor to alleviate oxidative stress [15]. Hence, a total polyphenol content of microalgae was measured using the Folin-Ciocalteu method. The result indicated that microalgae contained 360.4 µg/L-culture corresponding to gallic acid using vertical plastic-bag after 15 days cultivation, followed by horizontal plastic-bag with 289.6 µg/L-culture and plastic-bag with 230.6 µg/L-culture. On the other hand, the result of the reductive capability of microalgae was consistent with the total polyphenol. The microalgae of vertical plastic-bag had 413.4 µg/L-culture corresponding to ascorbic acid. Consequently, the microalgae cultivated by vertical plastic-bag had the highest total polyphenol and total reductive activity.

Table 1. The antioxidant detection of microalgal biomass under the condition of 46.7 h sunshine times and average 9.15 MJ/m².

|                | Corresponding to gallic acid \(^a\) (µg/L-culture) | Corresponding to ascorbic acid \(^b\) (µg/L-culture) |
|----------------|-----------------------------------------------|-----------------------------------------------|
| Plastic-bag    | 230.6                                         | 353.7                                         |
| Vertical plastic-bag | 360.4                                      | 413.4                                         |
| Horizontal plastic-bag | 289.6                                     | 373.2                                         |

\(^a\) The total polyphenol of microalgae was corresponding to gallic acid.

\(^b\) The total reductive capability of microalgae was corresponding to ascorbic acid.
4. Conclusions
Much attention has been directed toward the industrial production of algae. Several kinds of research have been reported on the application of algae for foods, biodiesel, bioalcohol, and pharmaceuticals. For bioenergy, cost-down and mass productions of algae are the focus of current research. In this study, three plastic-bag types were designed to explore the effect of mass transfer on microalgal production. Our result suggested that vertical plastic-bag presented higher biomass productivity than the other plastic types, especially when the weather and light were poor. Well growth of microalgae was also in response to better antioxidant activity detected by the total polyphenol and total reductive capability. Taking together, the vertical plastic-bag which was simply processed can be used to obtain better biomass productivity. This result had the potential to be applied in the industrial field.

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