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Full Length Research Paper

Biofixation of carbon dioxide by *Chlorococcum* sp. in a photobioreactor with polytetrafluoroethene membrane sparger

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The greenhouse effect, caused by excessive carbon dioxide emissions, constitutes a major aspect of global warming. Biological fixation of carbon dioxide using microalgae is an effective carbon dioxide reduction technology, but its widespread implementation is limited by the poor mass transfer efficiency. In this study, *Chlorococcum sp.* was cultured in a photobioreactor with a polytetrafluoroethene membrane sparger (PTFE) to study CO_2 biofixation and microalgae growth. Daily variations of dissolved oxygen (DO), pH and dissolved CO_2 were analyzed during batch culture of *Chlorococcum sp.* in the photobioreactor. The culture of *Chlorococcum sp.* under different operating conditions, such as pH, light cycle (light:dark) and nitrate feeding, were carried out to optimize the CO_2 biofixation rate and the algal productivity. The results confirmed that the photobioreactor with a membrane sparger is an alternative option for CO_2 removal from flue gas by cultivation of microalgae.

Key words: Bioreactor, polytetrafluoroethene membrane sparger (PTFE) membrane sparger, *Chlorococcum* sp., greenhouse gas.

INTRODUCTION

The excessive combustion of fossil fuel constituting a major aspect of global warming causes severe environmental destruction on the earth (IPCC, 2007). Currently, more than 80% of the energy produced globally each year is generated through the combustion of fossil fuels, which is the largest single CO_2 emission source (Korre et al., 2010; Sayre, 2010). Nearly one-third of anthropogenic CO_2 emission comes from combustion of fossil fuels in power plants worldwide (Mansourizadeh and Ismail, 2010). Emission trading and related CO_2 certificates have proven to be very costly for industries dependent on the burning of fossil fuels (Borkenstein et al., 2010). Biofixation of carbon dioxide can be achieved through the photosynthesis of terrestrial plants as well as photosynthetic microorganisms. Microalgae has a higher photosynthetic efficiency and biomass productivity, a faster growth rate and high added-value by-products, such as biofuel and feedstocks for animal, when compared with other photosynthetic plants (Gouveia and Oliveira, 2009; Sayre, 2010). Microalgae are a feasible and effective alternative for biofixation of carbon dioxide from the flue gas.

The CO_2 transfer efficiency is one of the most important parameters for enhancing the CO_2 biofixation rate and algal productivity in a photobioreactor culture system (Fan et al., 2007; Ryu et al., 2009). The carbon dioxide mass transfer capacity of a photobioreactor is determined by the liquid-phase mass transfer coefficient and the specific area available for mass transfer (Markl, 1977; Carvalho et al., 2006). For the purpose of mass transfer, the most frequently used approach is bubbling CO_2-enriched air into the bottom of the photobioreactor with diffusers (Kumar et al., 2010). However, the associated drawbacks are a loss of CO_2 to the atmosphere and poor

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Abbreviations: PTFE, Polytetrafluoroethene membrane sparger; **DO,** dissolved oxygen.

mass transfer rates because of the relatively low interfacial specific surface area and the low residence times of the gas in the culture (Cheng et al., 2006; Kumar et al., 2010). Another method for CO_2 mass transfer in a photobioreactor is using hollow fiber membranes. Compared with conventional gas dispersion methods, hollow fiber membranes have advantages of surprisingly high interfacial area provided by the membrane and the dramatic reduction in the amount of CO_2 lost to the atmosphere (Ferreira et al., 1998; Carvalho and Malcata, 2001). However, the hollow fiber membrane systems suffer from some drawbacks, including less turbulent hydrodynamic pattern, low mixing rate and biomass settling.

In this study, a photobioreactor with a polytetrafluoroethene membrane sparger (PTFE) membrane sparger, with the pore size of 0.22 μm, was specifically designed to explore the possibility of coupling CO_2 biofixation with microalgal growth. Artificial flue gas was used as feed gas with the composition of 10% CO_2, 5% O_2 and 85% N_2 to study the CO_2 biofixation rate and growth rate by *Chlorococcum* sp. in a photobioreactor. Operational conditions of the photobioreactor, such as pH, light cycles, nitrate feeding were investigated to maximize the CO_2 biofixation rate.

MATERIALS AND METHODS

Strain and culture medium

Chlorococcum sp. (FACHB-957) was provided by the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). The modified soil extract medium (SE medium) is composed of the following components (per liter): 250 mg $NaNO_3$, 75 mg $K_2HPO_4 \cdot 3H_2O$, 75 mg $MgSO_4 \cdot 7H_2O$, 25 mg $CaCl_2 \cdot 2H_2O$, 175 mg KH_2PO_4, 25 mg NaCl, 40 ml soil extracts, 5 mg $FeCl_3 \cdot 6H_2O$, 1 ml Fe-EDTA, 1 ml A_5 solution and 958 ml distilled water. Soil extract solution: 200 g soil without fertilization and 1000 ml of deionized water were placed in a 2 L bottle. The bottle with 100℃ water bath heating for 2 h cooled to room temperature and the procedure was repeated three times. The mixture of the bottle was left to settle and the supernatant collected and filtered. The A_5 solution was composed of the following (per 100 ml): 286 mg H_3BO_3, 181 mg $MnCl_2 \cdot 4H_2O$, 22 mg $ZnSO_4 \cdot 7H_2O$, 7.9 mg $CuSO_4 \cdot 5H_2O$ and 3.9 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$.

Photobioreactor system

The study of microalgae cultivation was carried out in an 8 L enclosed cylindrical glass photobioreactor with an inner diameter of 12 cm and a height of 75 cm, under the illumination of fluorescent lights. The aeration gas was passed through a bacterial gas filter prior to injection. The water was circulated through a water jacket surrounding the bubble column to control the temperature of the photobioreactor. The inflow gas was composed of 85% N_2, 5% O_2 and 10% CO_2 and was injected into the culture with 30 ml/min.

Three probes for dissolved CO_2, dissolved oxygen (DO) and pH were installed at the top of the photobioreactor. The dissolved carbon dioxide, pH and DO of the algal culture were monitored online and recorded every minute by the controlling system. Light was supplied by ten 40 W fluorescent lamps, uniformly placed outside the photobioreactor. The mixing of algal culture was achieved by using a peristaltic pump with a circulation rate of 500 ml min^{-1}. A schematic diagram of the experimental setup is shown in Figure 1a.

A membrane sparger with an internal aperture diameter of 0.22 μm that was made of PTFE was installed at the bottom of the photobioreactor. The parameters of the membrane sparger are presented in Table 1. A schematic diagram of the structure of the membrane sparger is shown in Figure 1b.

Measurement of light intensity

A light sensor (TES1332A, Taiwan) was used to measure the light intensity on the surface of the bioreactor. The average light intensity was calculated by taking the weighted average of 60 measurements, which are located at the three horizontal planes uniformly located along the vertical axis of the reactor, and 20 evenly distributed measuring points along the radial axis for each horizontal plane height of the reactor (Ryu et al., 2009).

Measurement of biomass concentration

The biomass concentration (g/L) was measured by analyzing the optical density of the cell suspension at an absorbance wavelength of 685 nm using a spectrophotometer (UV-Vis 2550, Shimadzu, Japan). A calibrated straight line was been obtained previously by plotting the A685 dry cell weight (g/L).

Analysis of CO_2 and O_2 concentration

The CO_2 and O_2 concentration in the influent gas and effluent gas was measured by a gas chromatograph (GC9160, Ouhua Technology, Shanghai) equipped with a thermal conductivity detector. A chromatography workstation (HW2000, China) combined with a data acquisition computer was connected to the gas chromatograph.

Measurement of the inorganic carbon (IC) and the total concentration of nitrogen sources (TN)

The IC and TN in the culture medium were measured by a TOC/TN analyzer (TOC-VCPN, Shimadzu, Japan). The culture medium was first filtered through a cellulose acetate membrane filter (0.22 μm) before it was used for IC and TN measurements.

CO_2 biofixation rate and CO_2 removal efficiency

The total carbon content of *Chlorococcum* sp. was measured by an elementar analyzer (Vario EL III, German). The oxygen production efficiency, the CO_2 biofixation rate and the removal efficiency can be calculated by the following equations:

$$CO_2 \text{ biofixation rate} = \frac{X_{max} - X_0}{t} \times \frac{C}{12} \times 44 \quad (1)$$

$$CO_2 \text{ removal efficiency} = \frac{Y_{in} - Y_{out}}{Y_{in}} \times 100\% \quad (2)$$

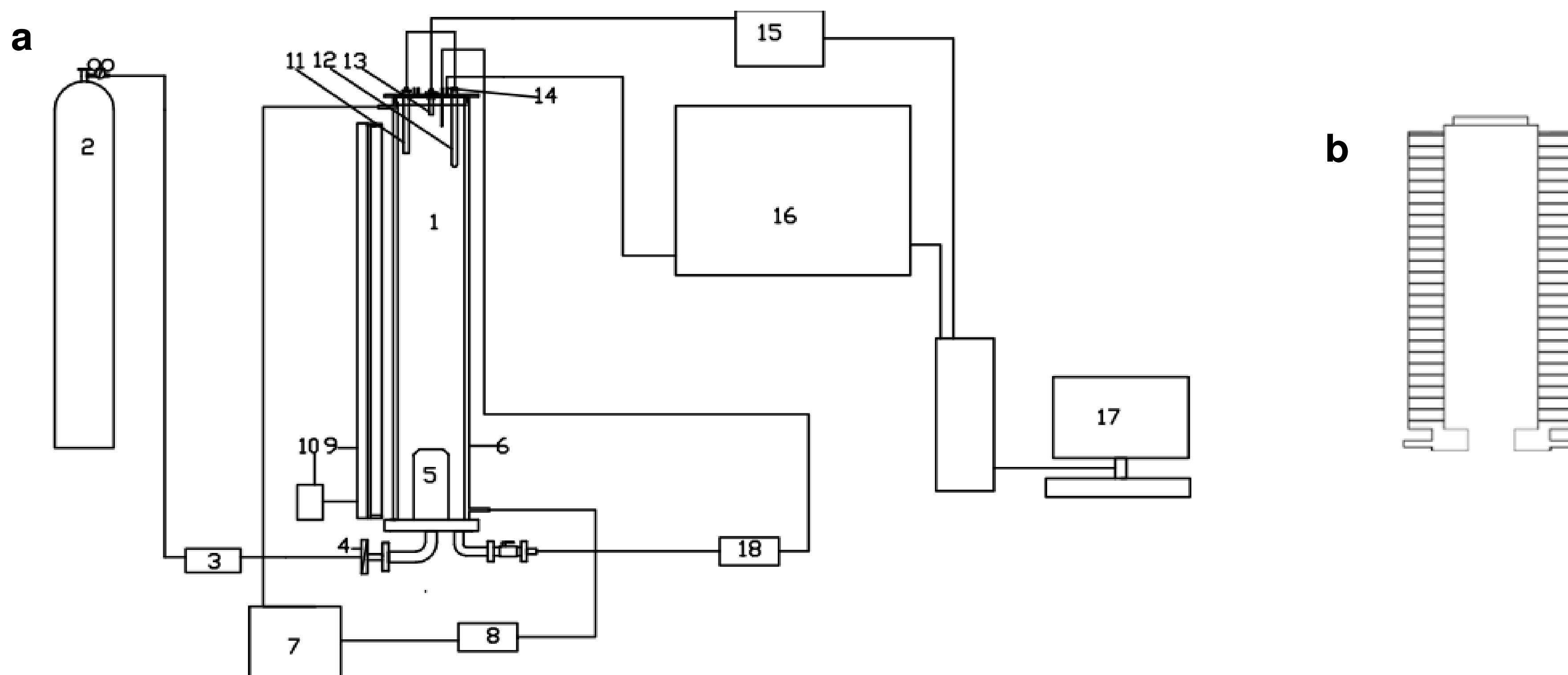

Figure 1. (a) Experiment setup of the airlift photobioreactor with an online process monitoring unit. 1, Bioreactor; 2, artificial flue gas cylinder; 3, flowmeter; 4, bacterial gas filter; 5, aeration membrane sparger; 6, water jacket; 7, thermostatic bath; 8,18-peristaltic pump; 9, light source; 10, microcomputer timer; 11, DO electrode; 12, pH electrode; 13, CO_2 electrode; 14, gas outlet; 15, signal translator; 16, gas chromatography; 17, computer. (b) Structure of the membrane sparger.

where, X_{max} and X_0 are the maximum cell concentration and the initial inoculated cell concentration (mg/L); t is the time required to reach the maximum cell concentration (d); C is the carbon content of dried biomass analyzed by the element analyzer; 44 is the molecular weight of CO_2, and Y_{in} and Y_{out} are the CO_2 molar fractions in the inlet and outlet gas phases, respectively.

The mass transfer coefficient for CO_2 ($K_La(CO_2)$)

The $K_La(CO_2)$ was determined in the microalgae culture with cells by a dynamic-state method according to the method use to measure $K_La(O_2)$ (Qi and Xia , 2004).

$$\frac{d[CO_2]}{dt} = K_La([CO_2]^* - [CO_2]) - R_{CO_2} \quad (3)$$

where, $[CO_2]^*$ is the equilibrium concentration of CO_2 in culture; $[CO_2]$ is the dissolved CO_2 concentration; R_{CO_2} is the CO_2 biofixation rate.

After injecting the gas, the $[CO_2]$ is monitored continuously with a peristaltic pump to make the culture homogenous. The CO_2 biofixation rate R_{CO_2} can be calculated as,

$$R_{CO_2} = -\frac{d[CO_2]}{dt} \quad (4)$$

When the concentration of dissolved CO_2 dropped below 1.0 mol/m^3, start to inject the inflow gas to the photobioreactor, and then the $[CO_2]$ begin to increase.

$$[CO_2] = -\frac{1}{K_La}\left(\frac{d[CO_2]}{dt} + R_{CO_2}\right) + [CO_2]^* \quad (5)$$

A straight line relationship between $[CO_2]$ and $\frac{d[CO_2]}{dt} + R_{CO_2}$ can be obtained. The negative reciprocal

Table 1. Parameters of the membrane sparger.

Parameter	Value
Pore size (μm)	0.22
Radius (cm)	7.0
Height (cm)	15.5

value of the slope is the K_La (CO_2).

RESULTS AND DISCUSSION

Characteristics of CO_2 biofixation by *chlorococcum* sp. during batch cultivation

Chlorococcum sp. was cultivated in the photobioreactor with a membrane sparger for six days. During the batch culture, after one day's preadaption, the algae exponential growth phase was observed from the second to the fourth day, followed by the stationary growth phase. Figure 2a shows that the dissolved CO_2, DO and the pH of the culture changed periodically with the illumination time and dark time during the batch cultivation.

The value of dissolved CO_2 coincided with the different photosynthetic efficiencies during the culture stages. The maximum and minimum values were 14.3 and 7.7 mM, respectively. The daily DO peak increased gradually with the increase of cell mass when the algae were in the exponential growth phase, and it decreased during the stationary phase. The O_2 production and carbon dioxide removal were closely related to the photosynthesis intensity of the algae in the culture. On the third day, the algae in the culture reached the highest photosynthetic intensity, and a maximum DO of 135% and a minimum dissolved CO_2 of 7.7 mM were obtained.

The pH value increased from 5.9 to 6.6 over the course of the culture time. The gradual increase of pH during batch culture could be attributed to the consumption of IC in the photosynthetic culture. The pH value increased and reached a maximum value at the end of the daily illumination period because the IC in the culture was utilized as carbon source by the algae (Jacob-Lopes et al., 2008).

The daily variations of O_2 and CO_2 concentration in the batch culture are shown in Figure 2b. The variations of O_2 and CO_2 in the outlet gas were consistent with the DO and dissolved CO_2 in the culture. The O_2 in the outlet increased as soon as the light was turned on at 8:00 in the morning and decreased sharply to a minimum following dark respiration at 20:00 in the evening.

The CO_2 concentration at the outlet decreased with the illumination time and increased gradually after the light was turned off. The O_2 decline and the CO_2 increase can be attributed to the extinction of photosynthesis without light. Moreover, higher O_2 concentration and lower CO_2 concentration were obtained with the increase in the photosynthetic intensity at the following day. The results suggest that O_2 production and CO_2 removal were observed during the illumination period, indicating that operational conditions should be optimized to photosynthesis intensity in order to enhance the CO_2 removal efficiency of the photosynthetic system.

Figure 2c shows the variation of CO_2 removal efficiency during batch cultivation. The CO_2 removal efficiency increased from 36 to 65% with the increase of cultivation time. A previous study (Chiu et al., 2008) reported a 20% CO_2 reduction in a semicontinuous photobioreactor with a high-density culture of *Chlorella* sp. with 10% CO_2 in air. The results of the present study indicate that a photobioreactor with a membrane sparger can provide a high level of dissolved CO_2 and high transfer efficiency. The high value of dissolved CO_2 and the long retention time sufficiently enhance the CO_2 biofixation rate and thus reduce the CO_2 flowing out of the photobioreactor.

Hydrophobic material and microbubbles produced by the membrane sparger make the CO_2 transfer from the gas phase into the liquid phase more easily in the culture. The mixing condition of the membrane sparger was improved compared with hollow fiber membranes. The relationship between $K_La(CO_2)$ and the aeration rate is shown in Table 2. The $K_La(CO_2)$ increase with the increasing aeration rate. High aeration rate contribute to low CO_2 removal efficiency and high $K_La(CO_2)$. When the aeration rate was above 0.004 vvm, dissolved CO_2 could maintain above 0.68 mM. It was reported that dissolved CO_2 above 0.68 mM would balance the demand for the photosynthesis of the microalgae cells (Becker, 1994). The total carbon input flux is determined by inflow rate and partial pressure of CO_2 (Carvalho et al., 2006). $K_La(CO_2)$ of 4-6 h^{-1} was required for a plate photobioreactor to meet the limiting CO_2 concentration in the liquid (Zhang et al., 2002). Regarding production cost and CO_2 removal efficiency, suitable K_La and aeration rate should be determined. In this study, $K_La(CO_2)$ of $0.52h^{-1}$ can satisfy the CO_2 demand of the photosynthesis in the culture.

Effect of pH on the growth of microalgae and the CO_2 biofixation rate

In this study, the pH was controlled by automatic addition of NaOH (4 M) solution and HCl (1 M) solution. Figure 3 shows the stable growth profiles of *Chlorococcum* sp. under pH 5.6, 8.0 and 10.0. As shown in Figure 3, the maximum cell concentration was found in the culture at pH 8.0 with a cell concentration of 0.61 g/L after five days. The growth rate at pH 10.0 was relatively low, and the cell concentration reached 0.17 g/L after four days of cultivation.

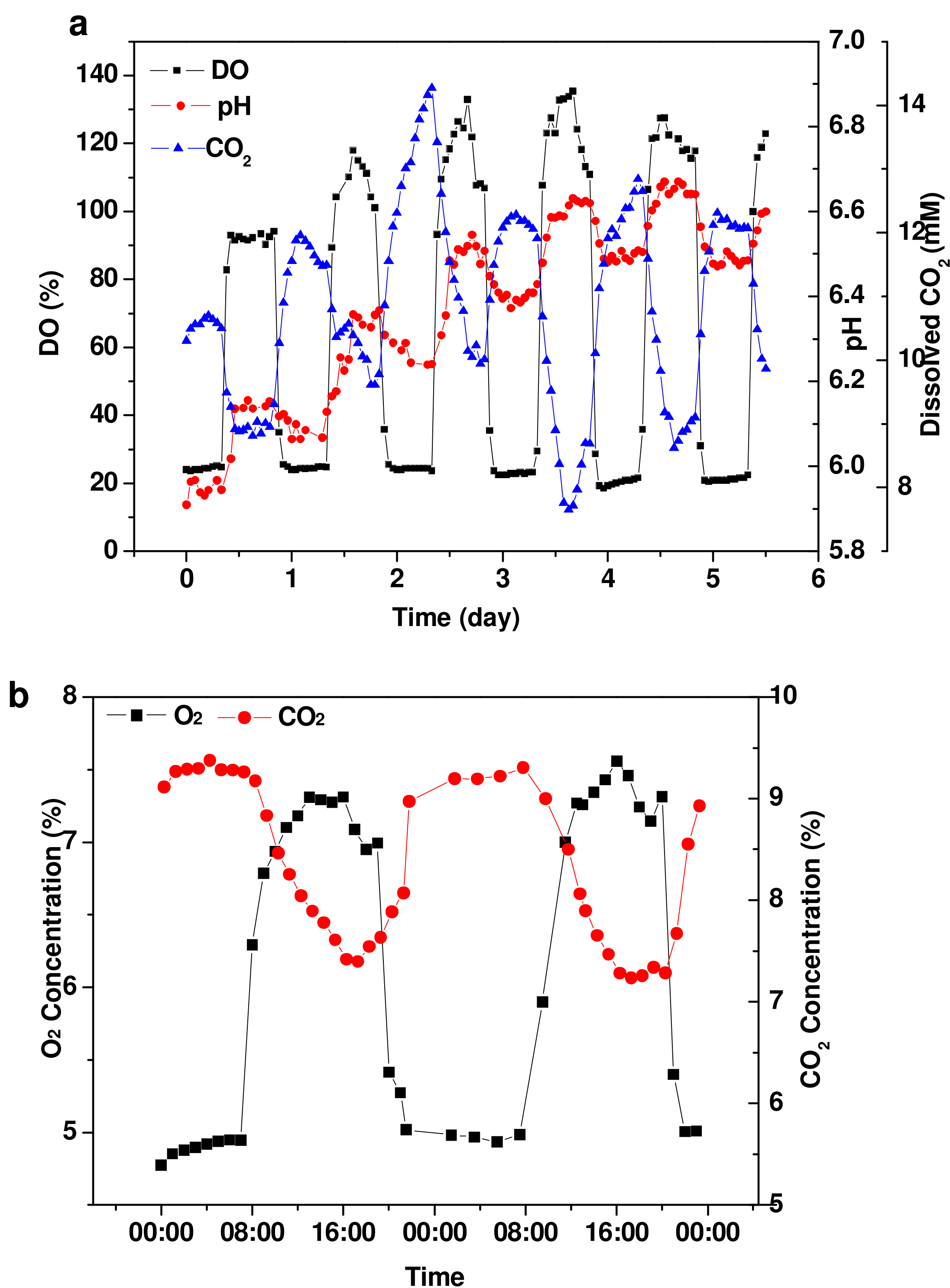

Figure 2. (a) Variation of pH, DO and dissolved CO_2 during batch cultivation; (b) daily variation of the O_2 CO_2 concentrations during batch cultivation; (c) variation of CO_2 removal efficiency during batch cultivation. DO, Dissolved oxygen.

According to an analysis of the elements in the *Chlorococcum* sp. cells at the end of cultivation, 1 g of biomass contained approximately 0.48 g carbon in the study. The value was used to estimate the CO_2 biofixation

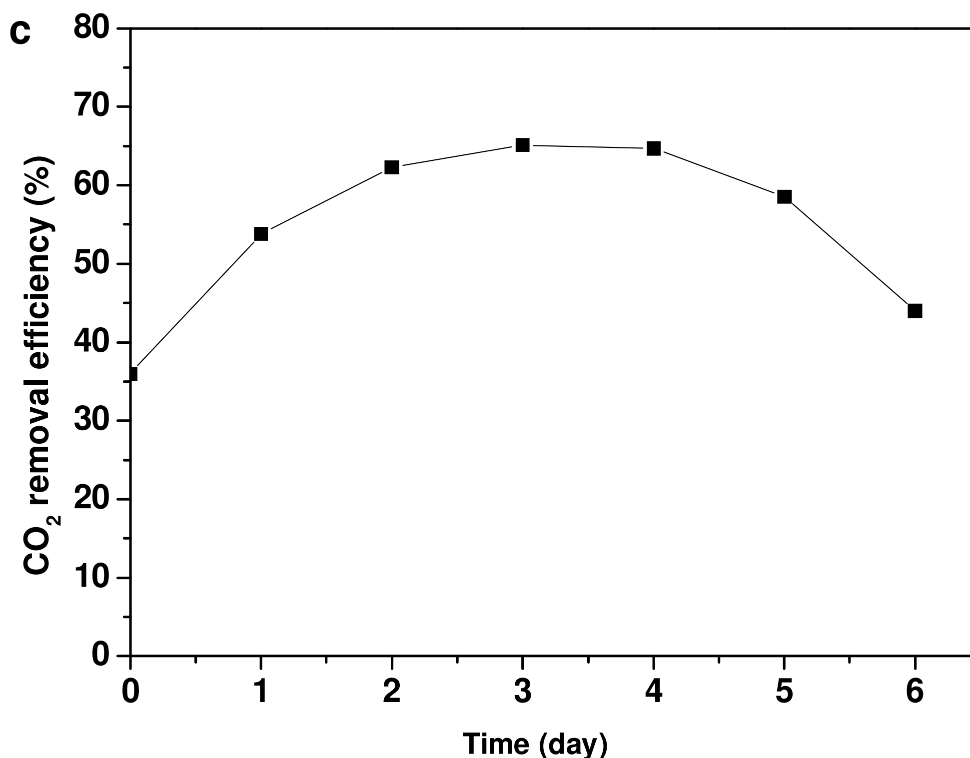

Figure 2. Contd.

Table 2. The relationship between $K_La(CO_2)$ and the aeration rate.

Aeration rate (vvm)	K_La (h^{-1})
0.013	0.52
0.025	0.84
0.038	1.62

rate. The CO_2 biofixation rate (mg /L /day) was calculated according to Equation 1, and the results are presented in Table 3. The highest CO_2 biofixation rate was 197 mg/L/day (pH 8.0). The lowest biomass concentration and CO_2 biofixation rate were observed when the pH was 10.0.

The pH affects the carbon concentration mechanism (CCM) in part by acting on the enzyme ribulose-1, 5-bisphosphate carboxylase. The activity of this enzyme is pH-dependent; its activity increases with an increase of pH (Jacob-Lopes et al., 2008). pH will influence the activity of ribulose-1, 5-bisphosphate carboxylase as well as the amount of inorganic carbon. Eukaryotic microalgae acquire IC from the surrounding aqueous medium to support photosynthesis (Colman et al., 2002). Most of microalgae species take up both CO_2 and HCO_3^-; few can use CO_3^{2-} as a carbon source. The concentration of IC increased with the culture time and exceeded 2000 mg/L at six days for a pH of 10.0. The predominant form of dissolved inorganic carbon is CO_3^{2-}, which is not available for microalgae to use for photosynthesis. More than 98% of the IC is in the form of HCO_3^- when the pH is 8.0, while the main form of the IC is CO_2 and H_2CO_3 when the pH is 5.6 (Clark and Flynn, 2000). This implies that microalgae at a pH of 8.0 will have a higher carbon fixation rate than they do at pH 5.6.

Effect of the light cycle on the growth of microalgae and the CO_2 biofixation rate

The light duration during the illumination time will affect the amount of energy received by the microalgae. The amount of light energy is closely related with the productivity in terms of the biomass and the cell growth rate, which consequently determines the photoauto-trophic carbon fixation capacity (Janssen et al., 2001; Jacob-Lopes et al., 2009). Figure 4 shows the growth profiles of the *Chlorococcum* sp. under different light cycles (day/night). Table 4 shows the CO_2 biofixation rate by microalgae under the various light cycles. The maximum biomass concentrations under light cycles of 24:0, 16:8 and 12:12 were 0.76, 0.95 and 0.62 g/L,

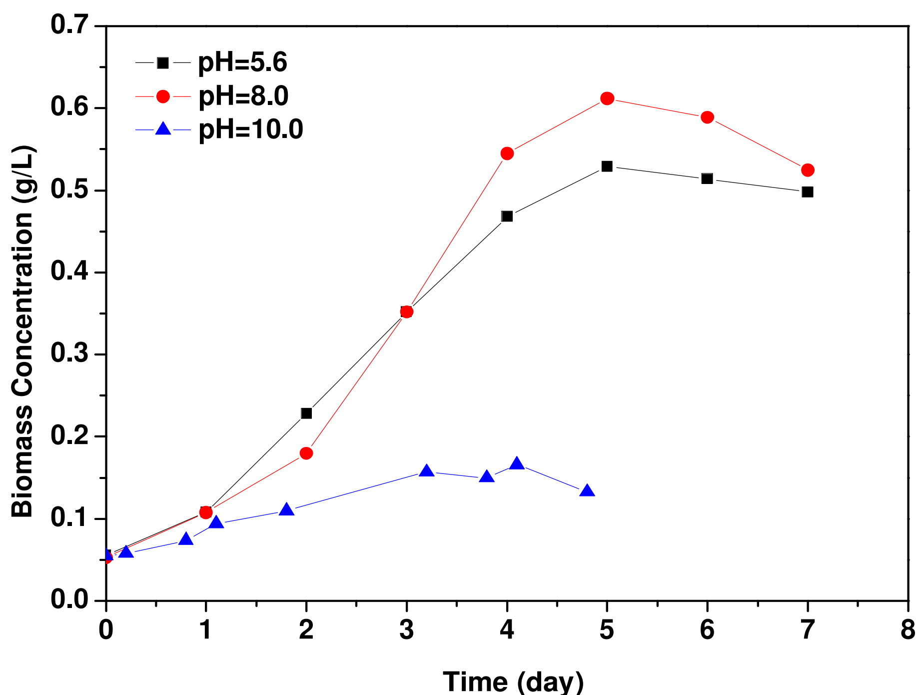

Figure 3. Growth of *Chlorococcum* sp. under various pH values.

Table 3. CO_2 biofixation rate for various pH values.

pH	Maximum cell conc. (g/L)	Biofixation rate (mg/L/day)
5.6	0.53	166
8.0	0.61	197
10.0	0.17	49

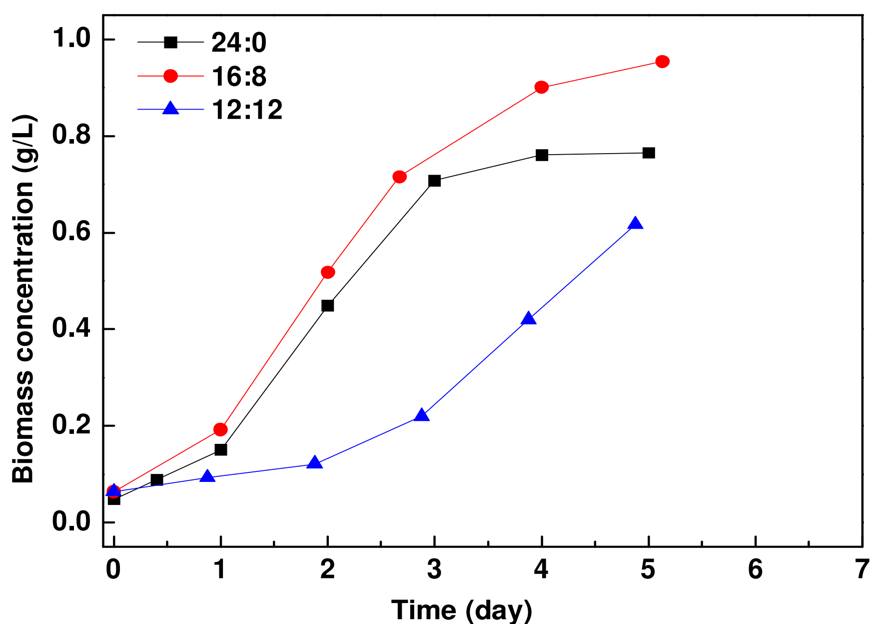

Figure 4. Growth of *Chlorococcum* sp. under the various light cycles.

Table 4. CO_2 biofixation rate under various light cycles.

Light cycle (light:dark)	Maximum cell conc. (g/L)	Biofixation rate (mg/L/day)
24:0	0.76	252
16:8	0.95	305
12:12	0.62	199

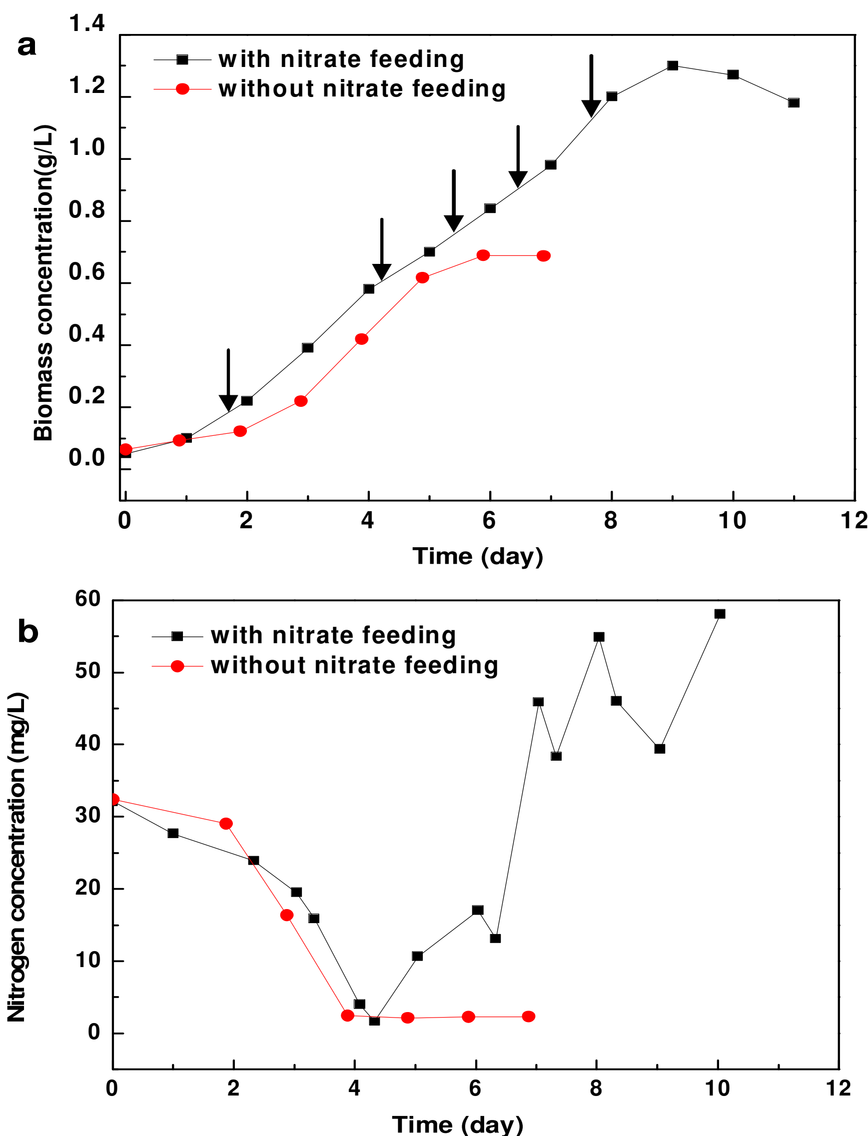

Figure 5. (a) Growth of *Chlorococcum* sp. with and without nitrate feeding (b) Variation of nitrogen concentration with and without nitrate feeding.

respectively. The corresponding CO_2 biofixation rates were 252, 305 and 199 mg/L/day, respectively. The maximum carbon dioxide fixation rate was obtained under the light cycle of 16:8.

Different cell growth profiles were seen as a function of the light periods. The cultures with light cycle of 16:8 (light: dark) showed similar characteristics to the algae cultivated with a continuous supply of light energy for the first 3 days. The maximum biomass concentration was observed at light cycle of 16:8. The culture with light cycle

of 24:0 obtained a lower cell concentration than that with16:8 at the end of the culture. The growth profile of 12:12 showed an adaption phase of two days before the exponential phase. The results show that supplying suitable light energy was a key factor for enhancing the growth of *Chlorococcum sp.* and the CO_2 biofixation rate.

Effect of nitrate feeding on the growth of microalgae and the CO_2 biofixation rate

The effect of the nitrate feeding was analyzed to confirm that nitrogen availability is the primary limiting factor for algal growth and CO_2 biofixation (Jin et al., 2006; Kumar et al., 2010). The CO_2 biofixation efficiency has a close relation with the growth phase of the microalgae, which declines precipitously when the cell growth phase reaches the stationary phase, followed by a death phase (Jin et al., 2006). Fig. 5a shows the growth profiles of *Chlorococcum sp.* with and without nitrate feeding. The nitrogen source decreased sharply from 32 mg/L to 3 mg/L in 4 days without nitrate feeding. The maximum biomass concentration and carbon biofixation rate were only 0.69 g/L and 1.1 gCO_2/L, respectively. Nitrogen source was added to the culture to avoid nitrogen deficiency and maintain the nitrogen concentration above the limiting level (Fig. 5b). The biomass concentration and the carbon biofixation with nitrate feeding were 1.9 times of the values without nitrate feeding. The maximum concentration was 1.3 g/L, and the biofixation rate was 2.1 gCO_2/L. In addition, the logarithm growth phase was prolonged by three days with nitrate feeding. The results suggest that photobioreactors with nitrate feeding can efficiently accelerate the CO_2 biofixation and algae growth rate.

Conclusion

The results indicate that it is an effective way to supply CO_2 by the PTFE membrane sparger during microalgal culture. The maximum CO_2 removal efficiency reached 65% at the third day of the cultivation. The optimal pH and light cycle for the microalgae growth was 8.0 and 16:8. The maximum biomass concentration and the CO_2 biofixation rate of light cycle 16:8 were 0.95 g/L and 305 mg/L/d, respectively. The logarithmic growth phase of the algae was prolonged by three days with nitrate feeding. The principle of this photobioreactor design will be of great interest for both algal cultivation and biofixation of CO_2 from flue gas.

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