Effect of carbon dioxide on biomass and lipid production of *Chlorella pyrenoidosa* in a membrane bioreactor with gas-liquid separation

Lei Zhang, Yong-Zhong Wang*, Shengwei Wang, Ke Ding

Key Laboratory of Biorheological Science and Technology (Chongqing University), Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400030, China

A B S T R A C T

In this work, an immobilized-cell biofilm photo-bioreactor, in which the CO_2-rich gaseous phase was separated from the nutrient-rich liquid phase by a polytetrafluoroethylene membrane, was proposed to enhance the adsorption of CO_2 by algal cells and the formation of biofilm on the membrane surface by reducing the disturbance from bubbles. The biomass productivity and biochemical composition, CO_2 removal efficiency, and fatty acid profile were measured to evaluate effect of CO_2 concentration on biomass and lipid production for biotechnological applications of the photo-bioreactor. The maximal biomass productivity (4.06 g/m²/d), lipid productivity (0.64 g/m²/d), and CO_2 removal efficiency (52.5%) were obtained at 3% CO_2 concentration, and C_{16}–C_{18} fatty acids in this cultivated biofilm accounted for 96.28% to 98.61% of the total fatty acids in all runs. The results indicate that the productivities of lipid and biomass were significantly improved using the immobilized-cell cultivation method.

1. Introduction

Algae biomass is currently considered one of the most promising resources for industrial applications, such as wastewater treatment, nutraceuticals, and biofuels [1–3]. In particular, microalgae have attracted worldwide interests due to their advantages in biofuel production, such as rapid growth, high biomass productivity, and high lipid content [4]. Investigations have shown that the fatty acids produced by microalgae are suitable for biofuel production. Nevertheless, the high cost of harvesting microalgae biomass results in a limited commercialization potential [5,6].

Suspension cultivation and immobilization cultivation are two cultivation methods of algal biomass production. In the suspension method, the open raceway pond and the closed photobioreactor (PBR) are the two main cultivation systems [7]. Although suspension cultivation of algal biomass in open raceway pond was low-cost, there exist some drawbacks, such as low biomass productivities, time-consuming cultivation, contaminations of native species, and high cost of biomass collection [8]. Alternatively, a closed PBR provides a high biomass concentration in the suspension cultivation mode as a result of the higher photosynthetic efficiency of the algal cells [9]. Moreover, the bioreactor performance can be further improved by optimizing operation conditions (e.g., light, temperature, salinity, pH) in the suspension culture mode. However, the shortcomings of the suspension cultivation

mode, such as the high cost of biomass collection and bubble disturbance to biomass growth, are prominent and hinder its commercial applications [10].

To circumvent these restrictions, the biofilm-based cultivation system has recently been proposed, in which algal cells attach to the substrate surface and form biofilm [11]. Immobilization of microalgae cells in the biofilm-based PBR mainly aims to reduce costs for harvesting biomass, and achieve higher biomass productivity [12]. Furthermore, the cultivation system of algal biofilm possesses many advantages such as enhancing CO_2 mass transfer, and increasing the capacity of algal species to adapt to the environment [12,13]. Additionally, relatively high efficiency of photo-conversion may be achieved in an algal biofilm system [14]. Some biofilm reactors have currently been reported, such as a revolving biofilm bioreactor and prototype-scale twin-layer photo-bioreactor [15,16]. Although inexpensive harvesting costs and increased biomass productivities can be achieved in these reported biofilm-based cultivation systems, disturbance from the gas bubbles inhibits the adsorption of CO_2 and growth of algal cells because of the shear stress of CO_2 bubbles, and this challenge has not yet been overcome. Moreover, the gas-liquid mass transfer resistance is relatively high in these traditional photo-bioreactors. That is, in such a traditional photo-bioreactor, the captured CO_2, which serves as the carbon source for photosynthesis, must dissolve into the liquid medium through diffusion across the gas-liquid

interface, which may decrease CO_2 utilization efficiency [17]. Therefore, when designing a biofilm bioreactor the characteristics of CO_2 mass transfer should be considered to improve CO_2 absorption efficiency of microalgae. For example, Gross et al. developed a rotating biofilm growth system for the attached microalgae growth [6]. In this system, algal biofilms were rotated between the liquid phase and gas phase, CO_2 was directly captured by biofilm cells without going through dissolving CO_2 in the liquid medium, as a result, the CO_2 utilization efficiency was improved. Currently, the large-scale applications of algae biofilm are mainly in wastewater treatment. Unsuitable supporting material, labor- and time-consuming operation, and high investment costs reduce the possibility to scaling up the system for biomass and lipid production by biofilms [18]. Despite the high biomass productivity in biofilm growth, the lipid content was lower than that of a planktonic culture. Therefore, in order to accelerate the industrialization of algae biofilm system, more research will be engaged on these aspects including screening of high-yield oil strains, control of nutrients, improvement of attached material properties, and optimization of reactor design [19].

Although there have been many literatures considering CO_2 effect on algae in suspension mode, little researches have been engaged on the attached biofilm growth. In addition, CO_2 concentration is an important parameter during algal biofilm formation. Fan et al. found the redirection of the central metabolism including distribution of the intracellular substance and energy of C. pyrenoidosa under different CO_2 concentrations [20]. Thus, a high biomass yield and a high lipid content can be achieved by optimizing CO_2 concentration.

Based on above mentioned, a biofilm cultivation system with gas-liquid separation, in which the gas phase is located on the bottom of the bioreactor to provide CO_2 and the liquid phase is located on the top for nutrients, was fabricated and run with different CO_2 concentrations. In this work, biomass and lipid productivity, CO_2 removal efficiency, biochemical compositions, and fatty acids were measured to evaluate the effect of the inlet CO_2 concentration on biomass and lipid production for biotechnological applications of the designed photo-bioreactor. The aim of this work was to improve the growth characteristics of algal biofilm through eliminating disturbance from CO_2 bubbles and decreasing the cost in harvesting the algal biomass.

2. Materials and methods

2.1. Microalgal strains and cultivation

C. pyrenoidosa FACHB-9 was purchased from the Institute of Hydrobiology, Chinese Academy of Science (Wuhan, China). Suspension in 500 mL BG11 culture medium in Erlenmeyer flask was cultivated for 3 days at 150 rpm and 30 °C in a shaker and with an illumination of 30 μmol photons $m^{-2} s^{-1}$ from fluorescent light.

The used culture medium was BG-11, which contained $NaNO_3$ 1500 mg/L, K_2HPO_4 40 mg/L, $MgSO_4 \cdot 7H_2O$ 75 mg/L, $CaCl_2 \cdot 2H_2O$ 36 mg/L, citric acid 6 mg/L, Trace metal solution 1 mL/L (Trace metal stock solution: $FeC_6H_5O_7 \cdot NH_4OH$ 6 g/L, Na_2EDTA 1 g/L, $MnCl_2 \cdot 4H_2O$ 1.81 g/L, $ZnSO_4 \cdot 7H_2O$ 0.222 g/L, $Na_2MoO_4 \cdot 2H_2O$ 0.39 g/L, $CuSO_4 \cdot 5H_2O$ 0.08 g/L, H_3BO_3 2.86 g/L).

2.2. Immobilized-cell cultivation system

The conception of the biofilm photo-bioreactor with the characteristics of gas-liquid separation was shown in Fig. 1. The biofilm reactor (0.2 m × 0.08 m × 0.03 m) was made of Polymethyl Methacrylate (PMMA) and divided by a layer of Polytetrafluoroethylene (PTFE) membrane into liquid chamber (on the top of the reactor) (0.16 m × 0.04 m × 0.01 m) and gas chamber (on the bottom of the reactor) (0.16 m × 0.04 m × 0.01 m). PTFE as a selectively permeable membrane, across which gas rather than water could pass by permeation, was supported by a glass grid to bear the gravity of liquid medium.

Both gas chamber and liquid chamber were arranged two holes, respectively, which employed as liquid/gas inlets and outlets. Fluorescent lambs were located above the bioreactor as light sources and an illumination intensity was obtained by adjusting the distance between the reactor and light source. The liquid medium containing algal seeds was guided into the liquid chamber by a peristaltic pump, and the CO_2 mixed with air was led into the gas chamber where CO_2 was permeated into liquid chamber across PTFE membrane. Thus, algal cells could attach on the surface of PTFE and form into biofilm under light illumination.

Before experiment, seed solution of C. pyrenoidosa was inoculated into BG11 culture medium to obtain the final biomass concentration of 128 mg/L, then, the inoculated culture solution was pumped into the liquid chamber of the bioreactor at a rate of 6 mL/min by peristaltic pump. CO_2 gas passed through gas chamber at the rate of 40 mL/min with different CO_2 concentrations (0.03%, 1%, 3%, 5% and 10%), and CO_2 as carbon source of the photosynthesis of algal cells attached to the PTFE surface diffused into biofilm across PTFE membrane. Illumination intensity was maintained at 46 μmol photons $m^{-2} s^{-1}$ and culture temperatures were set at 28 ± 2 °C. The whole experimental operation was composed of two steps: Cell attachment and biofilm growth. During 24 hour cell attachment stage, parts of algal cells in the inoculated culture medium attached on the surface of the PTFE, beyond incubation of 24 h, the culture medium was completely replaced by the fresh medium without inoculation. In the second step, the attached cells on the PTFE surface independently grew by utilizing nutrients from the fresh medium and capturing photons till the stable biofilm growth was observed. Each parameter experiment was carried out with 3 parallels in this work, three samples were taken per day for testing over a period of 7 days, and all data were the average value of three determinations.

2.3. Analytical methods

The biomass was harvested by scraping, and rinsed with distilled water, dried by vacuum freezer dryer (FD-1A-50, China) till constant weight, then weighed by an electronic analytical balance (EL204, China) to obtain the dried cell weight (DCW).

The biomass productivity $(g/m^2/d)$ was calculated using Eq. (1):

$$P = \frac{W_2 - W_1}{(t_2 - t_1)S} \tag{1}$$

where W_2 and W_1 were the dried cell weight (g) at time t_2 (h) and t_1 (h), respectively. S referred to the area of PTFE membrane covered by biofilm (m^2). t_1 refers to the time at inoculation and t_2 the time harvesting biofilm.

CO_2 concentration was measured by a gas chromatograph with FID detector (GC-210, China), and CO_2 removal efficiency was calculated according to Eq. (2):

$$CO_2 \text{ removal efficiency} = \frac{C_{in} - C_{out}}{C_{in}} \times 100\% \tag{2}$$

where C_{in} and C_{out} means CO_2 concentrations at inlet and outlet of the bioreactor, respectively.

Lipid productivity $(g/m^2/d)$ was obtained by Eq. (3):

$$P = \frac{L_2 - L_1}{(t_2 - t_1)S} \tag{3}$$

where L_2 and L_1 were the lipids weight (g) at time t_2 and t_1, respectively.

pH value of the culture media was detected by pH meter (PHS-3E, China). NO_3^- concentration was determined using diphenylamine as described previously [21]. 20 μL sample were mixed with 90 μL of diphenylamine solution (3.34 g diphenylamine in 1 L 14.4 M H_2SO_4) in a 96 well plate, then added 85 μL H_2SO_4 and stirred for 10 min at room temperature. The solution absorbance at 630 nm was determined by a microplate reader (iMark, Japan). Finally, nitrate concentration could