



# An efficient Photobioreactors/Raceway circulating system combined with alkaline-CO<sub>2</sub> capturing medium for microalgal cultivation

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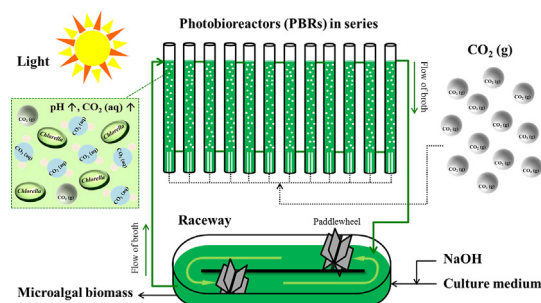
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## GRAPHICAL ABSTRACT



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## ABSTRACT

High efficiency of microalgal growth and CO<sub>2</sub> fixation in a Photobioreactors (PBRs)/Raceway circulating (PsRC) system combined with alkaline-CO<sub>2</sub> capturing medium and operation was established and investigated. Compared with a pH 6 medium, the average biomass productivity of *Chlorella* sp. AT1 cultured in a pH 11 medium at 2 L min<sup>-1</sup> circulation rate for 7 days increased by about 2-fold to 0.346 g L<sup>-1</sup> d<sup>-1</sup>. The maximum amount of CO<sub>2</sub> fixation and CO<sub>2</sub> utilization efficiency of *Chlorella* sp. AT1 could be obtained at a PBRs to Raceway ratio of 1:10 in an indoor-simulated PsRC system. A similar result was also shown in an outdoor PsRC system with a 10-ton scale for microalgal cultivation. Under the appropriate circulation rate, the stable growth performance of *Chlorella* sp. AT1 cultured by long-term semi-continuous operation in the 10-ton outdoor PsRC system was observed, and the total amount of CO<sub>2</sub> fixation was approximately 1.2 kg d<sup>-1</sup> with 50% CO<sub>2</sub> utilization efficiency.

## 1. Introduction

In the Paris climate agreement of 2015, “net zero emissions of carbon” was established as a long-term global goal between 2050 and

2100. However, the global average carbon dioxide (CO<sub>2</sub>) content continued to rise and increased to about 407 ppm in 2017, based on a report of the National Oceanic and Atmospheric Administration (NOAA). Therefore, sustained efforts to reduce CO<sub>2</sub> emissions are still needed

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globally. The biological carbon fixation of microalgae by photosynthesis is an environmentally friendly and sustainable-economy technology because natural sunlight can be used as energy for CO<sub>2</sub> fixation, and the produced biomass of microalgae can be applied in the development of products (Zhou et al., 2017). For example, the microalgal biomass could be used as a feedstock for biofuels and bio-refineries, such as biohydrogen, biogas, bioethanol, and biodiesel of energy products (Chiu et al., 2015; Gabrielyan et al., 2017). It also contains lutein,  $\beta$ -carotene, chlorophylls, phycobilins, and polyunsaturated fatty acids of high value products, such as feeds, cosmetics, and nutrient supplements (Chen et al., 2018).

For large-scale microalgal cultivation, the open raceway of microalgal cultivation system is mainly adopted because of low construction cost, simple operation and maintenance, ease to scale up, and relatively low energy consumption (Chisti, 2016; Rastogi et al., 2018). More than 90% of commercially global microalgal biomass is currently produced by open pond or raceway; however, there are the major drawbacks, such as microbial contamination risk and low biomass productivity (Shuba and Kifle, 2018). Therefore, most studies investigated whether to enhance microalgal growth rate in open raceway by equipment design (Yang et al., 2016a; Cheng et al., 2018; Sun et al., 2018; Zhang et al., 2018) and/or culture operation (Cheng et al., 2015; Chen et al., 2016; Yang et al., 2016b).

The growth of microalgae is strongly influenced by the availability of dissolved inorganic carbon in the culture medium for the cultivation period. The distribution of dissolved inorganic carbon species in aqueous solution is dependent on pH. When the pH of water is below 6.3 and ranges from 6.3 to 10, the dissolved CO<sub>2</sub> and HCO<sub>3</sub><sup>−</sup> are the dominant species, and both can be used for microalgal growth (Weiner, 2012). To enhance the microalgal growth, increasing the availability of dissolved inorganic carbon in the culture medium is necessary. An increase in dissolved inorganic carbon concentration in the culture medium by continuous CO<sub>2</sub> aeration and high CO<sub>2</sub> concentration supply is the general mechanism for enhancement of microalgal growth and has been demonstrated in most studies (Kao et al., 2014; Kuo et al., 2015; Cabello et al., 2017). However, the CO<sub>2</sub> utilization efficiency of microalgae is relatively low, because the amount of CO<sub>2</sub> content under continuous CO<sub>2</sub> aeration is often too high to be fully depleted by microalgae and is directly discharged into the atmosphere. Although CO<sub>2</sub> utilization efficiency of microalgae can be improved by intermittent CO<sub>2</sub> aeration, the growth of microalgae could be inhibited by an increase in pH of the culture medium (Nayak et al., 2013). To reduce CO<sub>2</sub> emission into the atmosphere, the biomass productivity and lipid productivity of microalgae were increased by adding sodium bicarbonate (NaHCO<sub>3</sub>) to enhance the concentration of dissolved inorganic carbon in the culture medium (Mondal et al., 2017; Nayak et al., 2018). However, the amount of NaHCO<sub>3</sub> that can be added to the culture medium is limited because of the increase in salinity by Na<sup>+</sup> accumulation. An optimal range of salinity in medium needs to be controlled because excessively high salinity inhibits the growth of microalgae (Zhu et al., 2016; Pandit et al., 2017). It is known that the concentration of dissolved inorganic carbon resulting from CO<sub>2</sub> gas dissolution is limited because of low solubility; however, in a high-pH medium, CO<sub>2</sub> gas dissolves simultaneously and neutralizes the OH<sup>−</sup> ions in the medium to generate HCO<sub>3</sub><sup>−</sup>, as follows: CO<sub>2</sub> (g) = CO<sub>2</sub> (aq) + OH<sup>−</sup> = HCO<sub>3</sub><sup>−</sup> (aq). Therefore, the biomass productivity of alkali-tolerant microalgae in high pH medium was increased because of higher dissolved carbon by CO<sub>2</sub> gas dissolution (Kuo et al., 2017; Vadlamani et al., 2017). However, the critical prerequisite for enhancement of microalgal growth by increasing the content of dissolved inorganic carbon in the alkaline medium is a requirement of alkali-tolerant microalgae. In our previous study, *Chlorella* sp. AT1 grew well at a broad pH range (6–11), with an optimal pH of 10 (Kuo et al., 2017).

The aim of this study was to establish an efficient Photobioreactors (PBRs)/Raceway circulating (PsRC) system composed of PBRs with CO<sub>2</sub> aeration and Raceway. The growth and CO<sub>2</sub> utilization efficiency of

*Chlorella* sp. AT1 cultured in an alkaline medium containing CO<sub>2</sub> captured content were evaluated. Moreover, an equation was established to predict biomass productivity and biomass production at different circulation rates in an indoor-simulated PsRC system. For outdoor cultivation in a PsRC system (around 10-ton scale), the stable growth performance of *Chlorella* sp. AT1 combined with a semi-continuous culture strategy is suitable for long-term microalgal cultivation.

## 2. Materials and methods

### 2.1. Microalgal cultures, media, and chemicals

The alkali-tolerant microalga *Chlorella* sp. AT1 used in this study was screened from N-methyl-N'-nitro-N-nitrosoguanidine (NTG) mutation of *Chlorella* sp. GD. The protocol of chemical mutagenesis and mutant isolation was performed based on our previous report (Kuo et al., 2017). The modified medium for microalgal cultures was composed of the following (per liter): 1.25 g of KH<sub>2</sub>PO<sub>4</sub>, 1.25 g of KNO<sub>3</sub>, 1 g of MgSO<sub>4</sub>·7H<sub>2</sub>O, 114.2 mg of H<sub>3</sub>BO<sub>3</sub>, 88.2 mg of ZnSO<sub>4</sub>·7H<sub>2</sub>O, 83.5 mg of CaCl<sub>2</sub>·2H<sub>2</sub>O, 49.8 mg of FeSO<sub>4</sub>·7H<sub>2</sub>O, 14.4 mg of MnCl<sub>2</sub>·4H<sub>2</sub>O, 10 mg of CuSO<sub>4</sub>, 7.1 mg of Na<sub>2</sub>MoO<sub>4</sub>, and 4 mg of CoCl<sub>2</sub>·6H<sub>2</sub>O. The pH of the medium was adjusted by NaOH addition, and the initial pH was set as pH 6.

### 2.2. Indoor-simulated PBRs/Raceway circulating (PsRC) system for microalgal cultivation

The indoor-simulated PBRs/Raceway circulating (PsRC) system was composed of 12 photobioreactors (4-L PBRs) in series, a 1,000-L Raceway, and a circulation pump. The 4-L PBR was a cylindrical glass column with a diameter and length of 8 and 100 cm, respectively. Gas was provided as 2% CO<sub>2</sub> mixed with ambient air. The microalgal cultures were aerated continuously with gas that was provided via bubbling from the bottom of the PBR with an aeration rate of 800 mL min<sup>−1</sup> (i.e., 0.2 vvm, volume of gas per volume of broth per min). The 1,000-L Raceway was a fiber-reinforced polymer (FRP)-made tank with a length, width, and height of 1.5, 1, and 0.7 m, respectively. The microalgal culture in the Raceway of indoor-simulated PsRC system was agitated by submerged pump to prevent microalgal cells stagnation. The circulation rate is the flow rate between PBRs and Raceway of PsRC system by driving circulation pump. The available light was provided on the top of Raceway by continuous cool-white fluorescent lights. The PsRC system was placed in a laboratory with the room temperature controlled at 26 ± 1 °C and with a surface light intensity of approximately 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

### 2.3. Inoculum preparation

The microalgal cells for inoculation were cultured in the PBR containing 4 L working volume of modified medium aerated with 2% CO<sub>2</sub> at an aeration rate of 0.2 vvm and cultured with about 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of light intensity at 26 ± 1 °C. The initial microalgal biomass concentration in fresh cultures was approximately 0.3 g L<sup>−1</sup>.

### 2.4. Batch cultivations in PBR and Raceway of PsRC system without circulation

To investigate the growth of *Chlorella* sp. AT1 in PBR and Raceway of the PsRC system at different levels of light intensity, the microalgal cells were cultured at light intensities of 200, 300, and 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  with 2% CO<sub>2</sub> at an aeration rate of 0.2 vvm and 26 ± 1 °C for 7 days. Subsequently, to evaluate the microalgal growth in alkaline medium, *Chlorella* sp. AT1 was cultured in PBR and Raceway with 2% CO<sub>2</sub> and air aeration at an aeration rate of 0.2 vvm in pH 6 and pH 11 media, respectively, at a temperature of 26 ± 1 °C and a light intensity of 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 7 days. The gas was provided by 6

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