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Maximizing CO_2 biofixation and lipid productivity of oleaginous microalga *Graesiella* sp. WBG-1 via CO_2-regulated pH in indoor and outdoor open reactors

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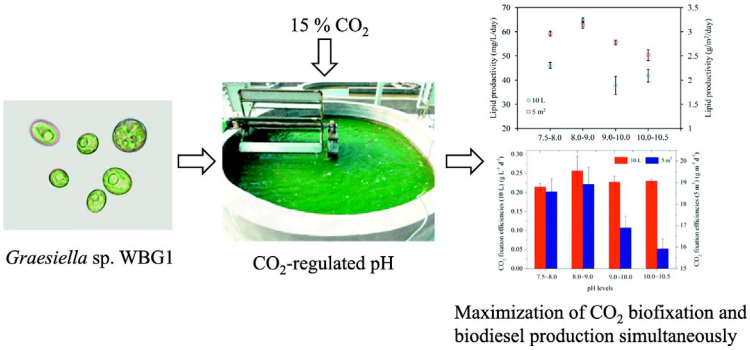
HIGHLIGHTS

- The lipid production of WBG1 under 4 CO_2-regulated pH levels was evaluated.
- The culture has a maximum CO_2 fixation rate under pH 8.0–9.0.
- Highest biomass, lipid content and productivity were obtained under pH 8.0–9.0.
- There is a positive correlation between CO_2 utilization efficiencies and pH values.
- Provide a strategy for coupling and maximizing the CO_2 fixation and lipid production.

GRAPHICAL ABSTRACT



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ABSTRACT

Carbon dioxide (CO_2) and pH are two interdependent factors that greatly impact the growth and lipid accumulation of microalgae. However, the effects of these two factors are usually studied separately. The use of exogenous CO_2, such as flue gas derived, to regulate pH in the large-scale cultivation of microalgae provides an ideal means for combining CO_2 biofixation and biodiesel production. In this study, the CO_2 biofixation and lipid production of oleaginous microalga *Graesiella* sp. WBG-1 was explored for four pH levels regulated by exogenous 15% CO_2 (flue gas concentration) in 10 L circular culture ponds and 5 m^2 open raceway reactors. Results revealed that pH 8.0–9.0 was the optimum pH for CO_2 fixation and lipid production, attaining the highest CO_2 fixation rates of 0.26 g L^{-1} day^{-1} and 18.9 g m^{-2} day^{-1}, respectively, lipid contents of 46.28% and 32.38%, and lipid productivities of 64.8 mg L^{-1} day^{-1} and 3.14 g m^{-2} day^{-1}. A positive correlation between CO_2 utilization efficiency and pH in open reactors was also suggested in this research, and thus provides direction for screening of CO_2 fixation by microalgae. The present study provides an excellent strategy for coupling CO_2 fixation and lipid production via microalgae in large-scale cultivation.

1. Introduction

The emissions of CO_2, a typical greenhouse gas, mainly derived from extensive fossil fuel consumption pose grave challenges to worldwide sustainability (Karl et al., 2009). Of the various approaches for capturing CO_2, biofixation by microalgae is considered one of the most promising

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technologies (Cheah et al., 2015; Kao et al., 2014). Microalgae can convert CO_2 to massive amounts of biomass and biological products efficiently through photosynthesis. Many microalgal strains with different tolerance to CO_2 have been isolated and studied (Cabello et al., 2017; Kassim and Meng, 2017; Solovchenko and Khozin-Goldberg, 2013; Zhao and Su, 2014); however, high cost and land demand greatly limit the biosequestration of CO_2 via microalgae (Lam et al., 2012). Another important application of these organisms, biodiesel derivation from microalgae, has been considered a potential renewable energy alternative to traditional fossil fuels for coping with energy shocks (Chisti, 2007; Hu et al., 2008; Mata et al., 2010). Unfortunately, this application faces the same difficulties as CO_2 biofixation does. Thus, combining biodiesel production with CO_2 biosequestration of microalgae to reduce costs has become an urgent issue whether for the biodiesel industry or to reduce carbon emissions (Kumar et al., 2010; Yen et al., 2015).

Though many species of microalgae have the ability to capture carbon under different concentrations of CO_2 owing to their unique carbon-concentrating mechanisms (CCMs) (Barber and Price, 2003) and therefore have relatively high CO_2 biofixation rates compared to terrestrial plant (Chen et al., 2013), the low CO_2 concentration in air seriously limits the metabolic activity and biomass productivity of microalgae, especially in large-scale cultivation. The addition of exogenous CO_2 is not only beneficial to carbon biofixation, but also beneficial to biodiesel production in microalgae culture. It has been reported that the synthesis and accumulation of lipids and TAGs in microalgae could be triggered by stress conditions, such as nitrogen starvation and osmotic stress (Brennan and Owende, 2010; Hu et al., 2008; Ördög et al., 2013; Zhao and Su, 2014), and enhanced by suitable concentrations of inlet CO_2 (Breuer et al., 2013; Moheimani, 2013). Using the CO_2 in flue gas as a carbon source for large-scale cultivation of oleaginous microalgae to reduce biodiesel costs and attain CO_2 fixation has been considered for microalgae biodiesel production (Yen et al., 2015).

For carbon biofixation and biodiesel production via microalgae, the close relationship between the inorganic carbon source represented by CO_2 and the pH values of the medium is particularly noteworthy. The pH of microalgal culture systems fluctuates throughout the day/night cycle because of the effects of photosynthesis and respiration. CO_2 consumption via photosynthesis raises pH values during the light period, and respiration at night lowers the pH. In the large-scale cultivation of oleaginous microalgae, the strong photosynthesis of microalgal cells can raise the pH to more than 10.0, which is obviously hazardous to the growth of most microalgal species (Oswald, 1988). pH usually has been studied as an independent factor regarding its effects on biomass production and lipid accumulation of microalgae in previous studies (Bartley et al., 2014; Breuer et al., 2013; Moheimani, 2013). Sodium bicarbonate, other chemical buffers, and hydrochloric acid are typically used to regulate and maintain the desired pH levels in culture systems in most studies, and CO_2 is rarely used (Bartley et al., 2014). However, the use of these chemicals to adjust pH is not only unsafe for the environment and does not easily control pH, but also further increases the biodiesel cost and is hence difficult to apply (Wang et al., 2011). Regulating and maintaining the pH of culture systems at an appropriate level for growth by injecting CO_2 is an optimum choice, whether considered from the perspective of cost or actual effect (Bartley et al., 2014; Han et al., 2013). Based on this, exogenous CO_2 and pH should be considered a combined factor in the combination of biodiesel production withCO_2 biosequestration via microalgae.

CO_2-based pH regulation of cultivation systems to optimum levels seems a good method for coupling biodiesel production and CO_2 fixation by microalgae, and maximizing CO_2 fixation and oil production of microalgae simultaneously should be of high interest. In the present study, the effects of four pH levels (7.5–8.0, 8.0–9.0, 9.0–10.0, and 10.0–10.5) (the suitable pH range for algae growth under natural conditions) regulated by exogenous 15% CO_2 (average concentration in flue gases) on the CO_2 fixation rates, growth, lipid contents, fatty acid profiles, and lipid productivities of the oleaginous microalga *Graesiella* sp. WBG-1 were explored. The aim of this study was to provide a valuable strategy for combining the goals of the algal biodiesel industry and that of reducing CO_2 emissions. The utilization efficiencies of injected CO_2 at different pH levels were also measured in these experiments, which was carried out in indoor circular culture ponds (10 L) and outdoor 5 m^2 raceway reactors (1000 L).

2. Materials and methods

2.1. Microalgae strains and medium

The oleaginous microalgal strain *Graesiella* sp. WBG-1, which exhibits excellent growth rate and lipid production and is suitable for large-scale cultivation, was used as experimental material. This strain was isolated from Chenghai Lake (N26°17′–26°28′, E100°38′–100°41′), which contains half-alkaline waters (nearly 1% alkalinity), located in Yunnan Province, China, and was deposited in the Algae Culture Collection of Wuhan Botanical Garden, Chinese Academy of Sciences (Wuhan, China). Modified BG11 medium (Wen et al., 2014) was used for the cultivation of *Graesiella* sp. WBG-1 both indoors and outdoors. The composition of this modified medium was similar to that in the literature except for the addition of 1 g L^{-1} $NaHCO_3$ and a concentration of 0.10 g L^{-1} $NaNO_3$. All the chemicals used for medium preparation were analytically pure (Sinopharm Group Co. Ltd., Shanghai, China). The culture medium was sterilized through autoclaving at 121 °C for 30 min in the laboratory. The water from Chenghai Lake was used to prepare the medium for outdoor culture after filtration and chemical disinfection, as described in our previous study (Wen et al., 2016).

2.2. Experimental photobioreactor and procedures

Self-designed circular culture ponds with culture volume of 10 L (indoor) and outdoor 5 m^2 open raceway reactors (1000 L) (Fig. 1) were used for the indoor and outdoor experiments, respectively. The 10 L reactors were manufactured from plexiglass and employed fluorescent lamps (30 W) to provide light. A water recycling system controlled by a thermostatic circulator (Hanon FCL6-20, China) was used to regulate the culture temperature. The steps and conditions of the indoor experiment are as follows. First, the WBG-1 cells were cultured to logarithmic

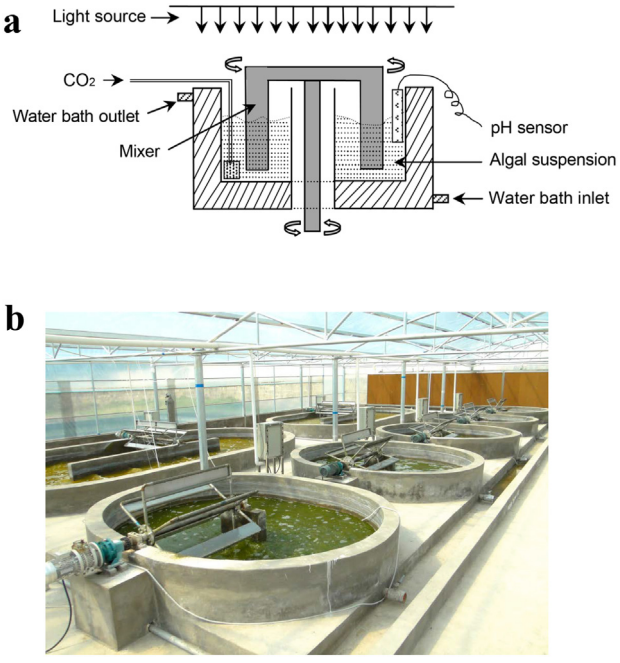

Fig. 1. Photobioreactors for microalgal cultivation. a: Scheme of 10 L circular culture pond; b: Photograph of 5 m^2 open raceway reactors.