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Optimization of CO_2 bio-mitigation by *Chlorella vulgaris*

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HIGHLIGHTS

- CO_2 fixation rate by *Chlorella vulgaris* was optimized.
- Growth parameters were affected by CO_2 concentration and aeration rate.
- Biochemical composition of algae did not change under different growth conditions.

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ABSTRACT

Biofixation of CO_2 by microalgae has been recognized as an attractive approach to CO_2 mitigation. The main objective of this work was to maximize the rate of CO_2 fixation (R_{CO_2}) by the green microalga *Chlorella vulgaris* P12 cultivated photoautotrophically in bubble column photobioreactors under different CO_2 concentrations (ranging from 2% to 10%) and aeration rates (ranging from 0.1 to 0.7 vvm). Results showed that the maximum R_{CO_2} (2.22 g L^{-1} d^{-1}) was obtained by using 6.5% CO_2 and 0.5 vvm after 7 days of cultivation at 30 °C. Although final biomass concentration and maximum biomass productivity of microalgae were affected by the different cultivation conditions, no significant differences were obtained in the biochemical composition of microalgal cells for the evaluated levels of aeration and CO_2. The present study demonstrated that optimization of microalgal cultivation conditions can be considered a useful strategy for maximizing CO_2 bio-mitigation by *C. vulgaris*.

1. Introduction

The increasing concentration of anthropogenic carbon dioxide (CO_2) in the atmosphere appears to be the major cause of global warming, which may have catastrophic consequences for the environment and the climate (Chiu et al., 2009). The amount of CO_2 in the atmosphere was 390.9 ppm in 2011, increasing on average 2 ppm per year for the past 10 years and reaching 140% of the pre-industrial level (280 ppm) (WMO, 2012). In order to reduce its atmospheric concentration, different abiotic (physical) methods have been evaluated, including injection into geological formations/deep oceans or utilization of absorbent materials (Kumar et al., 2010). These methods, however, require significant space of storage associated with elevated costs of monitoring, operation, and maintenance, raising serious concerns about potential CO_2 leakage over time (Bilanovic et al., 2009).

On the other hand, biological mitigation of atmospheric CO_2 has been deemed as a sustainable approach to physical methods (Kumar et al., 2011). Biofixation of CO_2 can be performed either by plants or photosynthetic microorganisms. Nevertheless, the process of CO_2 sequestration by plants can be viewed as an inadequate strategy of mitigation, since its contribution to CO_2 capture has been estimated to only 3–6% of fossil fuel emissions, mainly because of slow growth rates of terrestrial vegetation (Wang et al., 2008). Alternatively, microalgae have received renewed attention in recent years due to their faster growth rates and higher photosynthetic efficiency than terrestrial plants (Chiu et al., 2009; Dragone et al., 2011). These photosynthetic microorganisms can efficiently convert CO_2 from a point source into O_2 and biomass (Tang et al., 2011).

Microalgal biomass accumulates significant amounts of lipids, carbohydrates, proteins and other valuable compounds, such as pigments and vitamins, which can be used as active ingredients in pharmacy, food additives, feed supplements or in the production of biofuels (e.g. biodiesel, bioethanol, biohydrogen or methane) (Kumar et al., 2011; Milledge, 2011; Šoštarič et al., 2012).

Cultivation of microalgae has been exploited as an additional step in flue gas treatment, aiming the reduction of CO_2 levels in the exhaust flue gas. Previous studies have demonstrated that microalgae can be successfully employed for the treatment of simulated flue gases (Lee et al., 2000) or flue gases emitted from municipal waste incinerators (Douskova et al., 2009), coal-fired power plants (McGinn et al., 2011), industrial heater using kerosene as fuel (Chae et al., 2006) and gas boiler (Doucha et al., 2005).

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Moreover, microalgal species with high CO_2 fixation ability may minimize the significant costs of flue gas treatment (Ono and Cuello, 2007). In this regards, *Chlorella* species are considered as very promising candidates for the assimilation of CO_2, achieving CO_2 consumption rates between 0.73 and 1.79 g L^{-1} d^{-1} (Ho et al., 2011). In addition, it was found that CO_2 sequestration by *Chlorella* is not affected by volatile organic compounds present in the air stream (Keffer and Kleinheinz, 2002).

Since CO_2 assimilation by microalgae involves cell growth, the CO_2 fixation ability of microalgal species should positively correlate with their cell growth rate and biomass productivity (Ho et al., 2011). Thus, enhancing factors that influence biomass productivity is substantial to maximize CO_2 bio-mitigation (Tebbani et al., 2013).

Aeration rate is a key parameter to improve the growth of microalgal cells. Gas aeration in photobioreactors serves not only as a supply of CO_2 for cell growth, but also as a means of pH control, as well as for other important purposes such as provision of internal mixing to avoid nutrient concentration gradients, promotion of exposure of all cells to light (especially in high density cultures) to minimize self-shading and phototoxicity, and stripping of accumulated dissolved oxygen to reduce its toxicity to microalgae (Kumar et al., 2010).

The aim of this study was to maximize the CO_2 fixation by the green microalga *Chlorella vulgaris* P12 cultivated under different concentrations of CO_2 and aeration rates. It was also evaluated the effect of each culture condition on growth parameters and biochemical composition of microalgal cells. *C. vulgaris* P12 has been suggested as a promising feedstock for bioethanol production due to its ability to accumulate more than 40% of dry biomass as starch (Dragone et al., 2011).

2. Methods

2.1. Microorganism and culture conditions

The freshwater *C. vulgaris* (strain P12) was used for cultivation under photoautotrophic conditions. All experiments were carried out at 30 °C in 110 mL glass bubble columns photobioreactors containing 90 mL of medium, during 7 days. Agitation during cultivation of microalgae was provided by bubbling CO_2-enriched air through a needle (inner diameter of 0.8 mm) at the bottom of the photobioreactors. Different values of initial CO_2 concentration and aeration rates were used in the experiments (Table 1).

Illumination was provided by four fluorescent lamps (Sylvania Standard F18 W) on one side of the photobioreactors, at an irradiance level of 70 μmol m^{-2} s^{-1} measured by a LI-250 Light Meter with a LI-190 quantum sensor (LI-COR, USA).

The growth medium was prepared according to previous studies (Fernandes et al., 2010). The initial algal concentration was the same for all the cultivation conditions: 2.0×10^7 cells mL^{-1}.

2.2. Determination of microalgal cell concentration

Microalgal concentration in photobioreactors was measured by using an improved Neubauer hemocytometer. Biomass was also determined by cell dry weight after centrifugation of the sample at 8750*g* during 15 min, washing with distilled H_2O and drying at 105 °C until constant weight.

Table 1
Experimental range and levels of the independent process variables according to the 2^2 full-factorial central composite design.

Independent variable	Symbol	Range and levels		
		−1	0	+1
CO_2 concentration (%)	X_1	2	6	10
Aeration rate (vvm)	X_2	0.1	0.4	0.7

2.3. Determination of biomass productivity and specific growth rate

Maximum biomass productivity (P_{max}, g L^{-1} d^{-1}) was calculated from Eq. (1), where X_t was the biomass concentration (g L^{-1}) at the end of the cultivation period (t_x) and X_0 the initial biomass concentration (g L^{-1}) at t_0 (day).

$$P_{max} = (X_t - X_0)/(t_x - t_0) \quad (1)$$

Specific growth rate (μ_{max}, day^{-1}) was calculated from Eq. (2) according to (Abreu et al., 2012).

$$\mu_{max} = (\ln N_2 - \ln N_1)/(t_2 - t_1) \quad (2)$$

where N_1 and N_2 were the concentration of cells at the beginning (t_1) and at the end (t_2) of the exponential growth phase, respectively.

2.4. Determination of maximal CO_2 fixation rate

Maximal carbon dioxide biofixation rate, R_{CO_2} (g L^{-1} d^{-1}), was calculated from Eq. (3), as described by (Tang et al., 2011).

$$R_{CO_2} = C_C P_{max}(M_{CO_2}/M_C) \quad (3)$$

where C_C was the carbon content of microalgal cells (% w/w), measured by using a LECO CHNS-932 Elemental Analyser (USA), P_{max} was the maximum biomass productivity (g L^{-1} d^{-1}), M_{CO2} was the molar mass of CO_2 (g mol^{-1}) and M_C was the molar mass of carbon (g mol^{-1}).

2.5. Biochemical characterization of microalgal cells

Starch content of *C. vulgaris* was determined by enzymatic hydrolysis of the microalgal starch to glucose with α-amylase and amyloglucosidase, as previously described by Fernandes et al. (2012).

Total lipids were determined by the classic Folch chloroform-based lipid extraction protocol. The protein content of microalgal cells was quantified according to the method of Lowry. Contents of total lipids and proteins were expressed as a percentage per dry weight.

2.6. Experimental design and optimization by response surface methodology

The influence of initial CO_2 concentration and aeration rate (independent variables) on CO_2 biofixation rate (dependent variable) by *C. vulgaris* was assessed through a 2^2 full-factorial central composite design (CCD). For statistical analysis, the independent variables were coded according to Eq. (4), where each independent variable is represented by x_i (coded value), X_i (real value), X_0 (real value at the center point), and ΔX_i (step change value). The range and the levels of the variables are given in Table 1.

$$x_i = (X_i - X_0)/\Delta X_i \quad (4)$$

The experimental results were fitted with a second-order polynomial equation by multiple regression analysis. The quadratic mode for predicting the optimal point was expressed according to Eq. (5), where $\hat{y}_i$ represents the response variable, b_0 is the interception coefficient, b_i, b_{ii} and b_{ij} are the regression coefficients, n is the number of studied variables, and X_i and X_j represent the independent variables. Where possible, the model was simplified by elimination of statistically insignificant terms.

$$\hat{y}_i = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} X_i X_j \quad (5)$$