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Optimization of CO₂ bio-mitigation by *Chlorella vulgaris*



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HIGHLIGHTS

- CO₂ fixation rate by *Chlorella vulgaris* was optimized.
- Growth parameters were affected by CO₂ concentration and aeration rate.
- Biochemical composition of algae did not change under different growth conditions.

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ABSTRACT

Biofixation of CO₂ by microalgae has been recognized as an attractive approach to CO₂ mitigation. The main objective of this work was to maximize the rate of CO₂ fixation (R_{CO_2}) by the green microalga *Chlorella vulgaris* P12 cultivated photoautotrophically in bubble column photobioreactors under different CO₂ 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% CO₂ 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 CO₂. The present study demonstrated that optimization of microalgal cultivation conditions can be considered a useful strategy for maximizing CO₂ bio-mitigation by *C. vulgaris*.

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1. Introduction

The increasing concentration of anthropogenic carbon dioxide (CO₂) 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 CO₂ 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 CO₂ leakage over time (Bilanovic et al., 2009).

On the other hand, biological mitigation of atmospheric CO₂ has been deemed as a sustainable approach to physical methods (Kumar et al., 2011). Biofixation of CO₂ can be performed either by plants or photosynthetic microorganisms. Nevertheless, the

process of CO₂ sequestration by plants can be viewed as an inadequate strategy of mitigation, since its contribution to CO₂ 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 CO₂ from a point source into O₂ 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 CO₂ 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 CO₂ 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 CO₂, achieving CO₂ consumption rates between 0.73 and 1.79 g L⁻¹ d⁻¹ (Ho et al., 2011). In addition, it was found that CO₂ sequestration by *Chlorella* is not affected by volatile organic compounds present in the air stream (Keffer and Kleinheinz, 2002).

Since CO₂ assimilation by microalgae involves cell growth, the CO₂ 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 CO₂ 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 CO₂ 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 CO₂ fixation by the green microalga *Chlorella vulgaris* P12 cultivated under different concentrations of CO₂ 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 CO₂-enriched air through a needle (inner diameter of 0.8 mm) at the bottom of the photobioreactors. Different values of initial CO₂ 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 × 10⁷ 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

Table 1
Experimental range and levels of the independent process variables according to the 2² 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 |

at 8750g during 15 min, washing with distilled H₂O and drying at 105 °C until constant weight.

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 CO₂ 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).

$$R_{\text{CO}_2} = C_c P_{\max} (M_{\text{CO}_2} / M_C) \quad (3)$$

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_{CO_2} was the molar mass of CO₂ (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 CO₂ concentration and aeration rate (independent variables) on CO₂ biofixation rate (dependent variable) by *C. vulgaris* was assessed through a 2² 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.

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

The quality of the fitted polynomial model was expressed by the coefficient of determination R^2 , and its statistical significance was checked by the F -test. The significance of the regression coefficients was tested by t -value. Results were analyzed by the Experimental Design Module of the Statistica 8.0 software (Statsoft, USA). The model permitted evaluation of the effects of linear, quadratic and interactive terms of the independent variables on the chosen dependent variables. Each run of the experimental design was carried out in duplicate and the centre point condition was performed 6-fold.

Data were compared using one-way ANOVA followed by a Tukey's multiple comparison test with 95% confidence level.

3. Results and discussion

3.1. Effect of CO₂ concentration and aeration rate on microalgal growth

Final biomass concentration (X_{\max}) and maximum biomass productivity (P_{\max}) of *C. vulgaris* cultivated under different aeration rates and CO₂ concentration in the air stream are shown in Table 2.

It can be noted that *C. vulgaris* was able to grow under all the evaluated levels of aeration and CO₂; however X_{\max} and P_{\max} were significantly influenced by the cultivation conditions.

Regardless the CO₂ level in air, higher values of X_{\max} and P_{\max} were obtained under the aeration rate of 0.4 vvm when compared with those values obtained at 0.1 vvm. These results are in good agreement with a previous study, which reported that cell concentration and biomass productivity of *Chlorella* sp. AG10002 rose when aeration rate is increased from 0.1 to 0.4 vvm (Ryu et al., 2009). According to Fan et al. (2007), the gas–liquid mass transfer coefficient is strengthened by increasing the feed gas flow rate. Moreover, the higher turbulent motion of liquid intensifies the movement of cells at the region adjacent to the photobioreactor wall, leading to an enhanced use of light by microalgae.

On the other hand, X_{\max} and P_{\max} were almost similar when the aeration rate was increased from 0.4 to 0.7 vvm at 6% and 10% CO₂ concentrations. It has been suggested that high aeration air flux reduces the gas bubble retention time, releasing the gas mixture to the outside of the photobioreactor before an efficient mixing occurred (Fan et al., 2007); as a consequence, the majority of the supplied CO₂ might have not been efficiently used by microalgal cells. Therefore, for high CO₂ levels in the air flow (6% and 10% in this study), an increased release of CO₂ molecules might have occurred. Otherwise, for 2% CO₂ concentration, the release of CO₂ molecules from the photobioreactor when the aeration rate was increased from 0.4 to 0.7 vvm would have been lower than for 6% and 10% CO₂ levels.

The final biomass concentration and maximum biomass productivity of *C. vulgaris* were also significantly influenced by the

percentage of CO₂ in the air stream. It can be observed in Table 2 that X_{\max} and P_{\max} increased nearly 45% when CO₂ concentration was raised from 2% to 6% at 0.4 vvm. This result is compatible with previous research observations. For example, Ryu et al. (2009) showed that maximum cell concentration and biomass productivity of *Chlorella* increased from 1.78 to 2.02 g L⁻¹ and from 0.295 to 0.335 g L⁻¹ d⁻¹, respectively, by elevating the CO₂ level in air from 2% to 5%. It should be mentioned that besides employing a different microalgal strain (*C. vulgaris* P12), the medium composition used in the present study differed also from that (Allen medium) used previously (Ryu et al., 2009). These factors could explain the higher X_{\max} and P_{\max} values found in our work in comparison with those values obtained by Ryu et al. (2009).

It is worth mentioning that the dissolved inorganic carbon (DIC) in the culture medium exists in the forms of CO₂, H₂CO₃, HCO₃⁻ and CO₃²⁻, usually in an equilibrium, and represents the carbon source for microalgae growth (Tang et al., 2011). Therefore, the stimulatory effect of CO₂ (up to 6%) on growth parameters of *C. vulgaris* could be related to the increased availability of carbon as a result of the higher DIC concentration. Beyond that, CO₂ abundance influences several key enzymes in carbon metabolism, such as carbonic anhydrase and Rubisco. Thus, increasing CO₂ concentration can enhance the carboxylating activity and repress the oxygenating activity of Rubisco, resulting in increased photosynthesis (Yang and Gao, 2003). However, such hypothesis should be considered up to a certain level of CO₂ (6% in the case of the present study).

The use of higher CO₂ levels can also result in low pH. Decrease of pH will cause the decrease of the activity of carbonic extracellular anhydrase and inhibit cell growth (Tang et al., 2011). The lower values of X_{\max} and P_{\max} obtained under 10% CO₂ concentration in air in comparison with those found at 6% CO₂ level validate such hypothesis.

Among the different cultivation conditions tested, the highest values of final biomass concentration (10.0 ± 0.5 g L⁻¹), maximum biomass productivity (1.3 ± 0.0 g L⁻¹ d⁻¹) and maximum specific growth rate (0.95 ± 0.04 d⁻¹) of *C. vulgaris* P12 were all obtained at 6% CO₂ and 0.4 vvm.

Table 3
Elemental composition of *C. vulgaris* cells.

| Element | Content (wt.%) |
|---------|----------------|
| C | 45.6 ± 0.5 |
| H | 6.9 ± 0.1 |
| N | 2.7 ± 0.2 |

C = carbon.
H = hydrogen.
N = nitrogen.

Table 2
Growth parameters of *C. vulgaris* cultivated under different CO₂ concentrations and aeration rates at 30 °C.

| Cultivation condition | | Growth parameters | | Time (d) |
|-----------------------------------|---------------------|-----------------------------------|---|----------|
| CO ₂ concentration (%) | Aeration rate (vvm) | X_{\max}^a (g L ⁻¹) | P_{\max}^b (g L ⁻¹ d ⁻¹) | |
| 2 | 0.1 | 5.5 ± 1.7 | 0.7 ± 0.2 | 7.9 |
| | 0.4 | 6.9 ± 1.2 | 0.9 ± 0.2 | 7.7 |
| | 0.7 | 8.3 ± 2.8 | 1.1 ± 0.4 | 7.6 |
| 6 | 0.1 | 6.8 ± 0.5 | 0.9 ± 0.0 | 7.6 |
| | 0.4 | 10.0 ± 0.5 | 1.3 ± 0.0 | 7.7 |
| | 0.7 | 8.9 ± 0.8 | 1.2 ± 0.1 | 7.4 |
| 10 | 0.1 | 6.0 ± 1.9 | 0.8 ± 0.3 | 7.5 |
| | 0.4 | 8.6 ± 2.4 | 1.2 ± 0.3 | 7.2 |
| | 0.7 | 8.5 ± 0.1 | 1.2 ± 0.0 | 7.1 |

^a Final biomass concentration.

^b Maximum biomass productivity.

Table 4

Experimental matrix and results of carbon dioxide biofixation rate ($\text{g L}^{-1} \text{d}^{-1}$) with coded levels of CO_2 concentration (X_1) and aeration rate (X_2) according to the 2^2 full-factorial central composite design.

| Runs | Independent variables | | CO_2 biofixation rate ($\text{g L}^{-1} \text{d}^{-1}$) | |
|------|-----------------------|-------|--|-----------|
| | X_1 | X_2 | Experimental | Predicted |
| 1 | -1 | -1 | 1.15 | 1.10 |
| 2 | -1 | +1 | 1.87 | 1.79 |
| 3 | +1 | -1 | 1.35 | 1.55 |
| 4 | +1 | +1 | 1.92 | 2.10 |
| 5 | -1 | 0 | 1.50 | 1.69 |
| 6 | +1 | 0 | 1.93 | 2.07 |
| 7 | 0 | -1 | 1.54 | 1.64 |
| 8 | 0 | +1 | 2.08 | 2.26 |
| 9 | 0 | 0 | 2.10 | 2.20 |
| 10 | 0 | 0 | 2.07 | 2.20 |
| 11 | 0 | 0 | 2.18 | 2.20 |
| 12 | 0 | 0 | 2.29 | 2.20 |

3.2. Optimization of maximal CO_2 biofixation rate

In order to calculate the maximal carbon dioxide biofixation rate (R_{CO_2}), the elemental composition of *C. vulgaris* cultivated under varying growth conditions was determined. No statistically significant differences were found in terms of carbon, hydrogen and nitrogen content of cells cultivated under different CO_2 concentrations and aeration rates (data not shown). This result ratifies previous findings of (Tang et al., 2011), where the carbon content of *Chlorella pyrenoidosa* SJTU-2 was nearly 50%, without greatly changes under CO_2 concentrations between 0.03% and 50%. In our study, the carbon content of *C. vulgaris* was $45.6 \pm 0.5\%$ in average (Table 3).

R_{CO_2} was calculated according to Eq. (3), and the results are shown in Table 4.

It can be noted that R_{CO_2} varied under the different cultivation conditions. The highest values of R_{CO_2} were obtained when *C. vulgaris* was cultivated at 6% CO_2 concentration and 0.4 vvm.

Due to the differences observed among values, a statistical analysis was carried out aiming at identifying which independent variable had significant influence on CO_2 biofixation rate. The statistical significance of CO_2 concentration and aeration rate on the response variable (R_{CO_2}) is given in Table 5.

According to this analysis, the linear term of aeration rate as well as the quadratic terms of CO_2 concentration and aeration rate showed significant influence on CO_2 biofixation rate at 95% confidence level.

After identification of the terms affecting CO_2 biofixation rate, the experimental values were fitted to a second-order equation (Eq. (6)) obtained by multiple regression analysis. Despite the terms X_1 and X_1X_2 were not statistically significant at $p < 0.05$, they were maintained in the model equation to avoid a decrease in the value of the R^2 coefficient. The coefficients of the proposed equation are given below:

Table 5

Effect estimates, standard errors and t -test for CO_2 biofixation rate by *C. vulgaris* according to the 2^2 full-factorial central composite design.

| Variables and interactions | Estimated effects | Standard errors | t_{value} | p |
|----------------------------|-------------------|-----------------|--------------------|--------------------|
| X_1 | 0.227 | 0.101 | 2.237 | 0.067 |
| X_1^2 | -0.683 | 0.152 | -4.491 | 0.004 ^a |
| X_2 | 0.610 | 0.101 | 6.021 | 0.001 ^a |
| X_2^2 | -0.493 | 0.152 | -3.241 | 0.018 ^a |
| X_1X_2 | -0.075 | 0.124 | -0.604 | 0.568 |

X_1 = CO_2 concentration; X_2 = aeration rate.

^a Significant influence at 95% confidence level.

Maximal CO_2 biofixation rate ($\text{g L}^{-1} \text{d}^{-1}$)

$$= 0.27 + 0.30C - 0.02C^2 + 3.39A - 2.74A^2 - 0.03CA \quad (6)$$

$$R^2 = 0.94$$

where C represents the CO_2 concentration in air and A represents the aeration rate.

The quality of the quadratic fit was represented by the coefficient of determination R^2 . As can be noted, the model explains more than 90% of the dependent variable's variability ($R^2 > 0.90$). The value of R^2 obtained for Eq. (6) means that the quadratic model is able to represent values in the experimental region in a satisfactory manner. The values predicted by the model are displayed in Table 4 along with the observed values. Comparison of these data indicates that there is a good agreement between the experimental and predicted values for the proposed range. Therefore, the experimental factorial design and regression analysis were effective to identify the optimal conditions for maximum biofixation of CO_2 by microalgae under the different levels of CO_2 in air and aeration rates.

The relation between independent variables and R_{CO_2} can be best visualized by examining the surface plot presented in Fig. 1.

Fig. 1 clearly shows that CO_2 biofixation rate was not linearly increased when the process variables were increased, but there was an optimum point after which the use of higher CO_2 concentration and aeration rate did not improve R_{CO_2} . This is in agreement with the analysis presented in Table 5, which showed significant effect of the quadratic terms of both variables on R_{CO_2} . An estimate of the critical point revealed that 6.5% CO_2 and 0.5 vvm were the conditions able to maximize R_{CO_2} . Under these conditions the model predicts a rate of CO_2 fixation by *C. vulgaris* of $2.29 \text{ g L}^{-1} \text{d}^{-1}$. Assays for validation of this model were then performed under the established operating conditions and the obtained values of R_{CO_2} , X_{max} and P_{max} were $2.22 \text{ g L}^{-1} \text{d}^{-1}$, $9.97 \pm 0.05 \text{ g L}^{-1}$ and $1.33 \pm 0.02 \text{ g L}^{-1} \text{d}^{-1}$, respectively.

The results achieved in our study compare favorably with others reported in the literature. For example, the CO_2 fixation rate by *C. vulgaris* LEB-104 cultivated under 5% CO_2 concentration was $0.25 \text{ g L}^{-1} \text{d}^{-1}$ (Sydney et al., 2010). A maximum R_{CO_2} of $0.87 \text{ g L}^{-1} \text{d}^{-1}$ was obtained for *Chlorella* sp. UK001 using a gaseous mixture (CO_2 : O_2 : N_2 = 10: 3: 87 (v/v)) at a constant flow rate of 0.05 vvm (Hirata et al., 1996). The higher CO_2 fixation rate by *C. vulgaris* P12 than

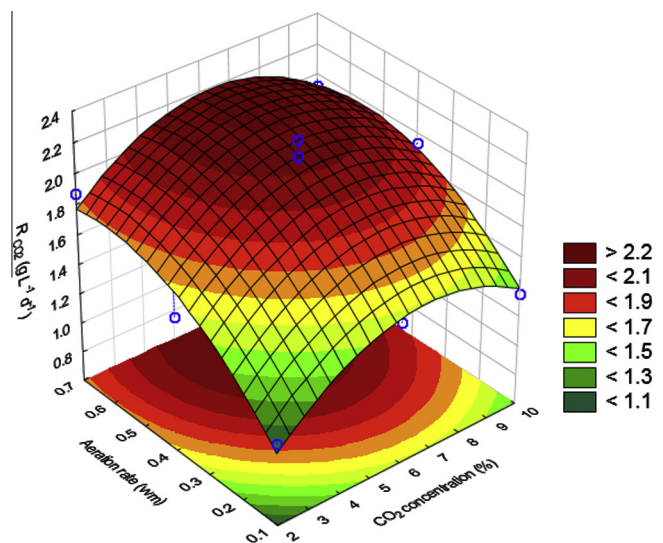


Fig. 1. Response surface of CO_2 fixation rate by *C. vulgaris* P12 as a function of CO_2 concentration in air and aeration rate.

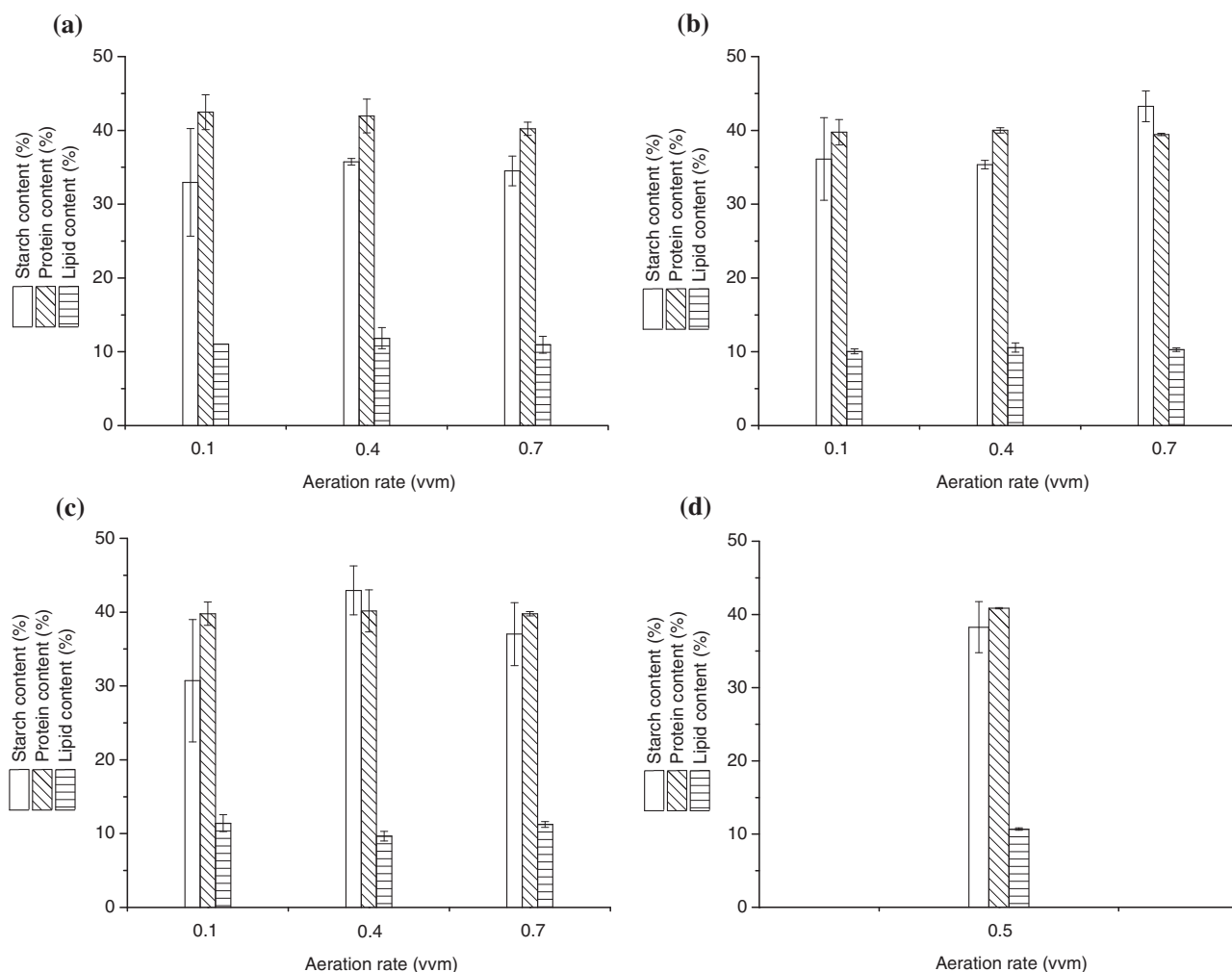


Fig. 2. Contents of starch, proteins and lipids of *C. vulgaris* cultivated in bubble column photobioreactors under different CO₂ concentrations (2% (a), 6% (b), 10% (c) and 6.5% (d)) and aeration rates.

those values obtained for *C. vulgaris* LEB-104 or *Chlorella* sp. UK001 could be mainly attributed to the microalgal cultivation conditions optimized in our study, which demonstrates the importance of selecting the process parameters in order to maximize R_{CO_2} . Additionally, a higher fixation ability of the *C. vulgaris* strain used in this work in comparison with those strains used in previous studies could also explain the variations observed in R_{CO_2} values.

3.3. Influence of CO₂ concentration and aeration rate on the biochemical composition of *C. vulgaris*

The content of starch, proteins and lipids of microalgae cultivated under diverse CO₂ concentration in air and aeration rates were determined and depicted in Fig. 2.

Despite variations in values of growth parameters and CO₂ fixation rates presented above, no statistically significant differences were observed in the biochemical composition of microalgal cells under varying growth conditions. Such behavior could be explained by mild conditions in terms of CO₂ concentration and aeration rates used in our study. It is known that only extreme/stressful cultivation conditions tend to promote changes in the accumulation of starch or lipids in *C. vulgaris* P12 cells (Dragone et al., 2011). Unfavorable culture condition, such as nutrient starvation has been intentionally used in order to enhance the lipid

content in *C. vulgaris* (Šoštarič et al., 2012). Therefore, two-stage cultivation processes, comprising a first stage to promote cell growth in nutrient replete conditions followed by a nutrient deficient stage to increase lipids/starch content have been suggested for large-scale production of biofuels from microalgae (Rodolfi et al., 2009). The conditions used in this study were far to be stressful to the cells, as demonstrated by the growth parameters presented in Table 2. Thus, a second stage under stressful cultivation conditions should be considered if microalgal biofuel production is aimed.

C. vulgaris P12 was able to accumulate about 37% starch, 41% proteins and 11% lipids under the evaluated cultivated conditions.

4. Conclusions

CO₂ fixation rate by *C. vulgaris* was maximized by the optimization of CO₂ concentration in air stream and aeration rate through a full factorial design.

This study constitutes an important step in the development of strategies to mitigate CO₂ in an environmentally sustainable manner by using a biological approach.

Future studies will be carried out in order to optimize simultaneously several other cultivation parameters (e.g. media composition, temperature, light intensity and mixing) but using

fractional factorial design to reduce the number of experimental runs.

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