Carbon dioxide mitigation by microalga in a vertical tubular reactor with recycling of the culture medium

Michele Greque de Morais¹*, Cleber Klasener da Silva¹, Adriano Arruda Henrard² and Jorge Alberto Vieira Costa²

¹Microbiology and Biochemistry Laboratory, School of Chemistry and Food, Federal University of Rio Grande, P.O. Box 474, Rio Grande, RS, Brazil.
²Biochemistry Engineering Laboratory, School of Chemistry and Food, Federal University of Rio Grande, P.O. Box 474, Rio Grande, RS, Brazil.

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Microalgae use photosynthesis as their principal metabolic mechanism to obtain organic carbon from the inorganic carbon contained in CO₂ using solar energy. This process releases oxygen into the atmosphere. The aim of this study was to determine the most appropriate biomass concentration for growing *Spirulina* sp. LEB 18 (Cyanobacteria) in a vertical tubular photobioreactor with biomass removal and recycling of the medium to maximize CO₂ biofixation. The maximum CO₂ biofixation was obtained when the culture was maintained at 600 mg L⁻¹ biomass concentration. Under this condition, the highest CO₂ biofixation value was 186.8 ± 73.1 mg L⁻¹ d⁻¹, and the maximum productivity was 85.9 ± 6.0 mg L⁻¹ d⁻¹.

**Key words:** Biofixation, carbon dioxide, microalgae, recycling, tubular photobioreactor.

**INTRODUCTION**

The increase in industrialization and urban population has led to a large demand by society for energy, which has increased the emission of atmospheric pollutants (CO₂, SOₓ, NOₓ, and other gases). According to NOAA (2013), atmospheric CO₂ has increased from 280 to 400 ppm over the last 215 years. The burning of fossil fuels for energy generation is the main source of the emissions of these pollutants.

Many alternatives have been studied to reduce CO₂ emissions into the atmosphere. One of the methods that is most suitable for reducing CO₂ emissions is the cultivation of microalgae. The photoautotrophic capability of these microorganisms converts CO₂ in biomass efficiently, minimizing environmental problems and the cost of carbon in the culture. Microalgae develop rapidly and can be grown in engineering systems, such as photobioreactors (Chiu et al., 2009). In addition to the ability to fix CO₂, the biomass of microalgae is rich in minerals, vitamins, lipids, pigments and proteins, with industrial and/or commercial applicability (Khan et al., 2009).

Microalgae are photosynthetic microorganisms that use...
inorganic carbon for growth and can be used for CO₂ mitigation. The process of mitigation by microalgae has many purposes: the capture of fossil carbon dioxide; the production of renewable energy with additional services to the environment (water treatment); and the generation of bioproducts (animal feed and fertilizers) (Kumar et al., 2010).

*Spirulina* is a filamentous cyanobacterium with a spiral format. Cyanobacteria are photosynthetic prokaryotic microorganisms that appeared more than 3 million years ago, forming the current atmosphere with oxygen, and since then have regulated the biosphere of the planet by removing CO₂ and releasing O₂ (Romano et al., 2000).

The photobioreactor configuration is one of the most important factors in controlling the biomass yield from photosynthetic cultures (Carvalho et al., 2011). The efficiency of light transmission per unit volume of culture, photosynthetic capacity, gas exchange and use of substrates are affected by the photobioreactor geometry and the homogenization of the culture medium with the added microalgae and gases, which are controlled by design and operation. To design a photobioreactor with an appropriate gas transfer system, the substrate must be effectively dissolved in the liquid medium to create a non-limitation situation for the cells (Pandey et al., 2014). The use of a vertical tubular photobioreactor increases the time that the gas remains in the medium, the area of contact between the light and culture, the photosynthetic rate, and consequently, the CO₂-use efficiency (Morais and Costa, 2007a). With microalgae cultures, recycling the medium allows the microalgae to use the nutrients until they are exhausted, with less exposure to the environment of the residues after biomass harvesting.

This study aimed to assess the most suitable biomass concentration in which to cultivate the Cyanobacteria *Spirulina* sp. in a vertical tubular photobioreactor, with biomass removal and the recycling of the medium, to obtain the maximum CO₂ mitigation.

**MATERIALS AND METHODS**

**Microorganisms and culture medium**

This study used the microalgae *Spirulina* sp. LEB 18 isolated from Mangueira Lagoon (33°30′12″S, 53°08′58″W). Zarrouk culture medium (Zarrouk, 1966) (Table 1) modified by Morais and Costa (2007b) was used to maintain the inoculum without the original carbon source of the culture medium (NaHCO₃). The inoculum was acclimatized with air mixed with 1% (w/w) CO₂ for 168 h with a 0.3 vvm flow rate.

**Culture conditions**

*Spirulina* sp. LEB 18 was grown in a 2 L (net volume 1.8 L, ø = 0.07 m) vertical tubular photobioreactor (VTPB) (Figure 1) at 30°C with a 12 h light/dark photoperiod. The 1200 Lux illuminance was provided by daylight-type fluorescent lamps (40 W).

Aeration was carried out by mixing compressed air with CO₂ through an industrial cylinder (White Martins - Brazil) with a flow rate of 0.3 vvm and a concentration of 12% (v/v) CO₂. The gas was added to cultures for 15 min every h during the light period (Morais and Costa, 2008).

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Amount (g L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaHCO₃</td>
<td>16.8</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.50</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>2.5</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>1.00</td>
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<tr>
<td>NaCl</td>
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</tr>
<tr>
<td>MgSO₄. 7H₂O</td>
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<tr>
<td>CaCl₂</td>
<td>0.04</td>
</tr>
<tr>
<td>FeSO₄. 7H₂O</td>
<td>0.01</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.08</td>
</tr>
<tr>
<td>Solution A5</td>
<td>1 mL</td>
</tr>
<tr>
<td>Solution B6</td>
<td>1 mL</td>
</tr>
</tbody>
</table>

Solution A5: (g L⁻¹): H₃BO₃: 2.86; MnCl₂.4H₂O: 1.81; ZnSO₄.7H₂O: 0.222; Na₂MoO₄.2H₂O: 0.390; CuSO₄.5H₂O: 0.079. Solution B6: (mg L⁻¹): NH₄VO₃: 22.86; KCr(SO₄)₂. 12 H₂O: 192; NiSO₄. 6H₂O: 44.8; Na₂WO₄. 2H₂O: 17.94; TiOSO₄. 8H₂O: 61.1; CO(NO₃)₃. 6H₂O: 43.98.

**Figure 1.** VTPB dimensions (mm).
of the duplicate tests. The cellular concentration was the cellular concentration (mg L$^{-1}$) at time $t$ (d), and $X_c$ (mg L$^{-1}$) is the cellular concentration at time $t_0$ (d) (Bailey and Olits, 1986).

A CHNS elemental analyzer (Perkin Elmer 2400, USA) was used to calculate the carbon content. The equipment was calibrated using the default cystine certificate (Perkin Elmer, USA). The results of cystine recovery, taken as a sample, were 100% for carbon (Baumgarten, 2010). The mean CO$_2$ fixation during cultivation was determined by the ratio between the sums of daily CO$_2$ fixation and the total number of days, according to the equation $BF = (X_R \cdot V_{FBR} \cdot m_{CB} \cdot m_{CO2} \cdot m_C \cdot t^1)$, where $X_R$ (mg) is the concentration of the produced biomass, $V_{FBR}$ (L) is the useful volume of the photobioreactor (PBR), $m_{CB}$ (mg mg$^{-1}$) is the mass fraction of carbon as determined in microalgal biomass, $m_{CO2}$ (mg mol$^{-1}$) is the molar mass of CO$_2$, $m_C$ (g mol$^{-1}$) is the molar mass of carbon, and time (d) is the time of cultivation.

**RESULTS AND DISCUSSION**

The maximum biomass production (365.4 ± 97 mg d$^{-1}$) was obtained when the culture was kept at a 600 mg L$^{-1}$ cellular concentration, followed by the culture being kept at 400 mg L$^{-1}$ cells (350.9 ± 43 mg d$^{-1}$) (Figure 2a).

Figure 2b and 3b show that maximum productivity rates (P) and maximum CO$_2$ fixation ($B_{CO2}$) were obtained in tests at cellular concentrations of 400 mg L$^{-1}$ (P = 97.3 ± 7.6 mg L$^{-1}$ d$^{-1}$, $B_{CO2} = 167.0 ± 18.5$ mg L$^{-1}$ d$^{-1}$) and 600 mg L$^{-1}$ (P = 85.9 ± 6.0 mg L$^{-1}$ d$^{-1}$ $B_{CO2} = 186.8 ± 73.1$ mg L$^{-1}$ d$^{-1}$), respectively. The total biomass removal was 1954 and 1945 mg L$^{-1}$ for cultures that kept at cellular concentrations of 400 and 600 mg L$^{-1}$, respectively.

The experiments that were performed at 200, 800 and 1000 mg L$^{-1}$ had total biomass removal values of 1517.9, 1567.9 and 1485.1 mg L$^{-1}$, respectively. According to Travieso et al. (2001), the ideal cell concentration in discontinuous processes for the maximum microalgal productivity is between 500 and 700 mg L$^{-1}$. In our study, the concentration of 600 mg L$^{-1}$ had the best results because it was the compensation, or saturation, point of photosynthesis. At this point, there is the maximum

**Analytical assessments**

Every 24 h, samples were collected aseptically to monitor cell concentration, which was calculated by measuring the optical density and dry weight of the biomass. The pH of the cultures was measured every 24 h with a digital pH meter (Quimis Q400H, Brazil) (Bailey and Olits, 1986).

**Kinetic parameters and carbon dioxide mitigation (CO$_2$)**

The kinetic parameters and CO$_2$ mitigation were calculated using the mean of the duplicate tests. The cellular concentration was measured daily, before and after biomass removal, and then used to calculate the concentration of produced biomass ($X_R$, mg), the productivity ($P$, mg L$^{-1}$ d$^{-1}$) and the mean CO$_2$ fixation ($BF$, mg L$^{-1}$ d$^{-1}$). The produced biomass was determined according to the equation $X_R = X_t \cdot V_R$, where $X_t$ (mg L$^{-1}$) is the cell concentration at time (d), and $V_R$ (L) is the volume of the medium that was withdrawn for the cultivation to return to the initial cell concentration value. The total biomass removal ($X_t$, mg L$^{-1}$) was calculated by totaling the daily removal of biomass. The productivity ($P$, mg L$^{-1}$ d$^{-1}$) was obtained according to the equation $P = (X_t - X_0) \cdot t^1$, where $X_t$ is the cellular concentration (mg L$^{-1}$) at time $t$ (d), and $X_0$ (mg L$^{-1}$) is the cellular concentration at time $t_0$ (d) (Bailey and Olits, 1986).

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photosynthetic efficiency because of the balance between the photosynthetic rate and respiration (Vonshak, 1997). Pelizer et al. (2003) obtained a maximum productivity of 76 mg L\(^{-1}\) d\(^{-1}\) in discontinuous cultivation that took place in a Raceway with an initial concentration of 100 mg L\(^{-1}\).

The photosynthesis process is the transformation of light energy into chemical energy in the form of ATP, NADPH, carbohydrates, proteins and lipids. The process is called photosynthesis because the coenzymes ATP and NADPH are used to add CO\(_2\) to organic molecules, featuring carbohydrate synthesis, where for each glucose molecule six fixed CO\(_2\) are generated (Alberts, 2010).

In cultures that were grown in an Erlenmeyer flask in semi-continuous mode with an initial concentration of 150 mg L\(^{-1}\), Reichert et al. (2006) obtained a maximum productivity (42.3 mg L\(^{-1}\) d\(^{-1}\)) in a test with a blended concentration of 500 mg L\(^{-1}\) and a renewal rate of 25%. No recycling of the medium took place in the Reichert et al. (2006) cultures; the amount removed was replaced by new medium, which provided rapid cell development. What differed with the tests that were presented in this study, which allowed the generation of higher yields compared to the aforementioned authors, was probably the use of a tubular photobioreactor. According to Morais and Costa (2007b), vertical tubular photobioreactors resulted in better fixation and kinetic results of CO\(_2\) compared to Erlenmeyer-type photobioreactors for Spirulina sp., S. obliquus and Chlorella vulgaris (Chlorophyta) when these microorganisms were supplemented with 0.038, 6, 12 and 18% CO\(_2\).

The advantage with recycling the culture medium is that it reduces costs because the nutrients are used until they are exhausted. However, some microalgae do not develop when this process is adopted. Nannochloropsis (Eustigmatophyceae) microalgae release auto-inhibitors during cultivation, affecting the culture’s development and limiting the recycling of the culture medium (Rodolfi et al., 2003).

Under all of the initial cell concentration conditions, the productivity of the tests decreased over time (Figure 2b). The cultures with cell removal provided the microalgae with the increased capacity to utilize the medium nutrients compared to cultures without removal because the microagal concentration is daily reduced to the initial concentration, thereby decreasing competition for nutrients. However, as new culture medium was not added, there was a reduction in the medium nutrients, which reduced the productivity over time. The consumption of nutrients decreases the osmotic pressure of the medium, which potentially affects the productivity (Poza-Carrion et al., 2001).

The cultures in which the biomass was removed until concentrations of 200, 800 and 1000 mg L\(^{-1}\) were reached had lower CO\(_2\) productivity and mitigation rates than did the cultures with concentrations of 400 and 600 mg L\(^{-1}\). The lower responses that were obtained for the yields and rates of mitigation of CO\(_2\) may have been caused by the phenomenon of photoinhibition in cultures that were maintained at the cellular concentration of 200 mg L\(^{-1}\) and by photolimitation in cultures with concentrations of 800 and 1000 mg L\(^{-1}\).

Photoinhibition is a phenomenon that occurs in the cultivation of microalgae because of a low cellular concentration in the culture, which allows a high incidence of light in the cells (Vonshak, 1997). In photolimitation, dense cultures of cells can block the penetration of light in the culture, limiting growth (Carvalho et al., 2011). In the dark, the rate of CO\(_2\) mitigation is negative because of cell respiration, which interferes with the process of photosynthesis and consequently the rates of mitigation of CO\(_2\) and microalgal growth. The flow of light that is emitted to cause photoinhibition or photolimitation depends on the genus and species of the studied microalgae.

Photosynthesis occurs in two distinct phases: a light phase (photochemical step) and a dark phase (chemical step). In the photochemical phase, radiant energy excites the photosynthetic pigments, and this state of excitation (energy) is transferred with the aid of water until the molecules NADP and ATP (chemical energy) are produced. The primary products of the photochemical step are ATP and NADPH. In this step also the release of oxygen occurs, as a byproduct of dissociation of the water molecule. In the chemical stage, the carbon from a molecule of CO\(_2\) is absorbed by a series of enzymatic reactions using the energy that is stored in the ATP and NADPH molecules, eventually forming the first product of photosynthesis, carbohydrate (CH\(_2\)O) for carbon skeletons (Cox and Nelson, 2014).

The Zarrouk cultivation medium for Spirulina microalgae in its original composition had a pH between 10.5 and 11.0. At the beginning of cultivation, the pH of all of the cultures was approximately 7.0 because the carbon source of Zarrouk medium (NaHCO\(_3\)), which is responsible for the alkaline pH, was replaced by 12% CO\(_2\). When Westerhoff et al. (2010) replaced the standard carbon source of the culture medium by CO\(_2\), they also found a reduction in the pH in microalgae cultivation. To avoid this reduction in pH Kumar et al. (2011), suggest the use of buffered systems. The highest-concentration nutrient in Zarrouk medium is sodium bicarbonate (16.8 g L\(^{-1}\)), constituting 40 to 50% of the total nutrient cost (Doucha et al., 2005), which means that if microalgae are used to fix CO\(_2\), there will be a reduction in the cost of nutrients in the culture and in the environmental problems that are caused by this gas.

According to Vonshak (1997), the optimal pH for the development of Spirulina is between 9.5 and 10.5. In cultures of Spirulina sp. LEB 18, with the removal of biomass and the recycling of the medium, the pH of the cultures varied between 6.83 and 9.11. Although the pH of the culture was within the optimum pH levels that were suggested by Vonshak (1997), there was no cell death.
Figure 3. (a) The pH as a function of time for the cultivation of *Spirulina* sp. LEB 18 with the removal of biomass to return the cell concentration to 200 mg L$^{-1}$ (●), 400 mg L$^{-1}$ (■), 600 mg L$^{-1}$ (▲) and 800 mg L$^{-1}$ (∆). (b) The mitigation of carbon dioxide as a function of the cellular concentration.

The experiments under different conditions studied showed daily increases in pH until 88.8 h, followed by decreases at 112.8 and 136.8 h, peaking at 160.8 h and mildly decreasing in the last h of cultivation (Figure 3a). This pH behavior was consistent with the daily production of biomass, which increased until 88.8 h, followed by a decrease in the consecutive 48 h (Figure 2a and 3a). The culture with a cell concentration of 600 mg L$^{-1}$ had the maximum production of biomass and a higher pH than that of the other cultures. The change in pH affects the solubility and bioavailability of nutrients, the transport of substrates through the cytoplasmic membrane, the enzyme activity and the transport of electrons for photosynthesis and respiration (Poza-Carrion et al., 2001).

**Conclusion**

When the microalga *Spirulina* sp. LEB 18 was cultivated in a vertical tubular photobioreactor with cell removal and medium recycling at a cellular concentration of 600 mg L$^{-1}$, the maximum CO$_2$ mitigation was reached. Under these conditions, the values that were obtained were 85.9 ± 6.0 mg L$^{-1}$ d$^{-1}$ biomass productivity and 186.8 ± 73.1 mg L$^{-1}$ d$^{-1}$ CO$_2$ fixation. Therefore, combining the cultivation of microalgae, the fixation of CO$_2$ and the recycling of the medium can lead to a reduction in the problems caused by the emission of this gas and in the expenses with culture medium and to the generation of biomass that can be used to obtain different products.

**Conflict of interests**

The authors did not declare any conflict of interest.

**ACKNOWLEDGMENTS**

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