Effect of light cycles (night/day) on CO₂ fixation and biomass production by microalgae in photobioreactors

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ABSTRACT

The objective of this study was to evaluate the effect of the photoperiod on the biomass production and carbon dioxide fixation rates using a photosynthetic culture of the cyanobacterium Aphanothece microscopica Nägeli in bubble column photobioreactors. The cultures were carried out at temperatures of 35 °C, air enriched with carbon dioxide at concentrations of 15% and photon flux density of 150 μmol m⁻² s⁻¹. The light cycles evaluated were 0:24, 2:22, 4:20, 6:18, 8:16, 10:14, 12:12, 14:10, 16:8, 18:6, 20:4, 22:2 and 24:0 (night/day), respectively. The results obtained indicated that the duration of the light periods was a determinant factor in the performance of the photobioreactors. A linear reduction in biomass production and carbon dioxide fixation rates with reductions in the duration of the light period was evident, with the exception of the 12:12 (night/day) cycles. Reductions of up to 99.69% in the carbon-fixation rates as compared with cultures under continuous illumination were obtained.

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1. Introduction

Various research projects are underway involving the application of cyanobacteria and microalgae in carbon dioxide biofixation, aiming to project viable photobioreactors for CO₂ sequestration technology [1–3]. Photosynthesis is a process comprising two steps, light reactions that only occur when the cells are illuminated; and carbon-fixation reactions, also known as dark reactions, that occur both in the presence and absence of light. Thus, in the first step the cells transform light energy into chemical energy, which is stored in high-energy compounds for later use in the carbon-fixation reactions [4]. The use of these photosynthetic pathways in environmental engineering processes requires the use of solar energy so as to develop clean technology processes [5]. Thus the cells use the light energy by way of exergonic reactions, producing energy that is used in the synthesis of compounds as from carbon dioxide fixation by way of endergonic reactions [6]. However, one of the operational problems of this type of technology refers to the lack of availability of light energy for whole time periods.

The light regimes to which the cultures are submitted are considered to be an important factor in the productivity and yield of photosynthetic reactions [7,8]. Various studies have been carried out focused on the effect caused by different photon flux densities incident on photobioreactors, but few reports can be found on the effects of the duration of the day and night cycles [9–11]. Thus a comparison of different photoperiods is necessary in order to determine the most efficient light regimes for industrial purposes.

Thus the objectives of the present study were to evaluate the effect of the photoperiod on biomass production and carbon dioxide fixation rates by the cyanobacterium Aphanothece microscopica Nägeli in bubble column photobioreactors.

2. Materials and methods

2.1. Microorganism and culture medium

Unialgal cultures of Aphanothece microscopica Nägeli (RSMan92) were originally isolated from the Patos Lagoon estuary, Rio Grande do Sul State, Brazil (32°01’S–52°05’W). Stock cultures were propagated and maintained on synthetic BGN medium [12], with the following composition (g/L): K₂HPO₄ 3H₂O (0.040), MgSO₄ 7H₂O (0.075), EDTA (0.001), H₃BO₃ (2.860), MnCl₂ 4H₂O (1.810), ZnSO₄ 7H₂O (0.222), Na₂MoO₄ 2H₂O (0.390), CuSO₄ 5H₂O (0.079), CaCl₂ 6H₂O (0.040), NaNO₃ (150), G₃H₆O₂₇H₂O (0.006), ammonium iron citrate (0.006), pH 8.0. The incubation conditions used were 25 °C, photon flux density of 15 μmol m⁻² s⁻¹ and a photoperiod of 12 h.
2.2. Photobioreactor design

Measurements were made in a bubble column photobioreactor. The system was built in 4-mm thick glass, an internal diameter of 7.5 cm, height of 75 cm and nominal working volume of 3.0 L. The dispersion system for the reactor consisted of a 1.5 cm diameter air diffuser located in the centre of the column. The reactor was continuously illuminated with 16 20 W fluorescent daylight-type tubes (General Electric, Brazil) connected in parallel, located in a photoperiod chamber. The duration of light cycles was controlled by timer. Airflow into the photobioreactor was provided via filtered air and pure CO\textsubscript{2} cylinder through Teflon tubing. The CO\textsubscript{2}/air mixture was adjusted to achieve the desired concentration of carbon dioxide in the airstream, through three rotameters that measured the flow rates of the carbon dioxide, the air and the mixture of gases, respectively.

2.3. Obtaining of the kinetic data in an experimental photobioreactor

The experiments were carried out in bioreactors operating in a batch mode, fed with 3.0 L synthetic BGN medium. The experimental conditions were the following: initial cell concentration of 0.1 g L\textsuperscript{-1}, isothermal reactor operating at a temperature of 35 °C, photon flux density of 150 \mu\text{mol} m\textsuperscript{-2} s\textsuperscript{-1} and continuous aeration of 1 VVM with the injection of air enriched with 15% carbon dioxide. Such conditions were previously defined by Jacob-Lopes et al.\[13\]. The light cycles evaluated were 0:24, 2:22, 4:20, 6:18, 8:16, 10:14, 12:12, 14:10, 16:8, 18:6, 20:4, 22:2 and 24:0 (night:day), respectively. In light cycles of 24:0, the cell concentration was measured every 12 h during the growth phase of the microorganism. The tests were carried out in duplicate and the kinetic data referred to the mean of four repetitions.

2.4. Kinetic parameters

The volumetric growth rate was determined from the variation in cell concentration in a determined time interval, as shown in Eq. (1):

\[ \dot{X} = \frac{X_1 - X_0}{t_1 - t_0} \]

where \(X_0\) and \(X_1\) are the biomass concentration at times \(t_0\) and \(t_1\), respectively. In the present study, \(t_0\) and \(t_1\) were zero and 156 h, respectively. In light cycles of 24:0 (night:day), the \(t_1\) considered was 48 h.

The carbon dioxide fixation rate was evaluated from the relationship between the carbon content of the cells and the volumetric growth rate of the microorganism, as shown in Eq. (2):

\[ R_C = \frac{C_{\text{CO}_2}}{X_0} \left( \frac{M_{\text{CO}_2}}{M_C} \right) \]

2.5. Analytical methods

The cell concentration was evaluated gravimetrically by filtering a known volume of culture medium through a 0.45 \mu\text{m} filter and drying at 60 °C for 24 h. The photon flux density was determined using a photometric (Spectronics, model XRP3000), measuring the light incident on the external reactor surface. The temperature was controlled using thermostats, and measured using a polarographic probe (Mettler Toledo, InPro5000 series). The flow rates of the carbon dioxide, air and CO\textsubscript{2} enriched air were determined using rotameters (AFSG 100 Key Instruments). The composition of the elements of the \textit{Aphanocystis microscopica Nageli} cells was determined using a PerkinElmer 2400 CHN/O element analyser. Two milligrams of biomass were oxidised at 1000 °C and the resulting gases were determined using a thermal conductivity probe for carbon. The standard used was acetanilide, with a composition of 71.09% carbon, 11.84% oxygen, 6.71% hydrogen and 10.36% nitrogen.

3. Results and discussion

In photosynthetic cultures, the amount of light energy received and stored by the cells has a direct relationship with the carbon-fixation capacity, consequently determining the productivity in biomass and cell growth rate. In nature, light energy is available in a discontinuous way, since the light varies from day to night. Such considerations are relevant in carbon sequestration processes in photobioreactors, since the viability of these systems requires the use of solar energy for photosynthesis. Systems of this type, fundamentally based on natural resources, are highly affected by the lack of availability of light energy during whole periods of time. Fig. 1 shows the variation in growth of the cyanobacterium \textit{Aphanotheca microscopica Nageli} under conditions of 35 °C, 150 \mu\text{mol} m\textsuperscript{-2} s\textsuperscript{-1} and 15% CO\textsubscript{2} with a continuous source of light energy.

An analysis of the growth curve shows the lack of an adaptation phase for the microorganism, with exponential growth occurring as from 12 h of cell residence time. The stationary phase was observed as from the sixth day of culture. The maximum cell concentrations obtained were 5 × 100 g/L, representing a more than 50-fold increase in cell density as compared to that initially present in the reactor.

Fig. 2 shows the growth curves for the cyanobacterium \textit{Aphanotheca microscopica Nageli}, with light cycles reduced at 2 h intervals. Different cell growth profiles can be seen as a function of the duration of the light periods. The cultures grown under photoperiods of 2:22 (night:day) showed characteristics similar to those grown with a continuous supply of light energy, whilst those grown in the absence of light showed evidence of limited carbon source for cell growth, since the cyanobacteria are unable to use inorganic carbon sources in the absence of light, and the organic carbon concentrations in the culture medium were insufficient for the energy maintenance of respiratory metabolism.[14]

These results are more evident in Table 1, which presents the kinetic characterisation of the growth and carbon fixation in the biomass by \textit{Aphanotheca microscopica Nageli}, under the different light cycles evaluated.

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From the analysis of variance (ANOVA) and Tukey’s test ($p < 0.05$), maximum volumetric productivity was shown to occur under conditions with a constant supply of light energy (0:24), the values not differing significantly ($p = 0.41$) from those obtained in experiments carried out with photoperiods of (2:22), suggesting that the supply of light for periods greater than 22 h did not influence the volumetric growth rate. These results demonstrate that Aphanothece microscopica Nägeli is capable of storing sufficient energy to sustain cell growth for periods of up to a maximum of 2 h in the dark, without affecting the rate of photosynthetic metabolism. For the other photoperiods evaluated, all the values for volumetric growth rate differed statistically ($p < 0.0001$). Similar results were obtained for the maximum cell densities, for which the photoperiods (0:24) and (2:22) (night:day) were statistically equal ($p < 0.05$). On the other hand, it was shown there were no significant differences in the maximum cell concentrations for the cultures grown with dark periods greater than 18 h.

The influence of the light cycles has been reported as a determinant factor in photosynthetic activity and in the growth rates of microalgae in photobioreactors [15–17]. According to these authors, light is a limiting substrate in these systems, which are affected by light/dark zones that depend primarily on the configuration, agitation and mixture in the reactor, associated with the possibility of cultures with discontinuous periods of light energy supply. Additionally, cell concentration is another parameter which determines the availability of light in photobioreactors. As result of the mutual shading occurring at high cell densities, the light intensity within the reactor becomes also a function of the biomass concentration [18]. As a result, the cells are exposed to different light intensities, with a considerable effect on system performance.

The pronounced variations in the volumetric growth rates and maximum cell density as a function of the duration of the light cycle showed that the cell concentration decreased proportionally with the fraction of time that the microorganism was exposed to intermittent light conditions as compared to continuous illumination. An exception to this behaviour was shown with photoperiods of 12:12 (night:day), in which higher productivity

![Fig. 2. Growth curves in different light cycles (dark:light).](image-url)
mentally on the access to light energy, which should be provided with the light availability. [20,21], suggesting the dependence of the biomass composition cell constituents, but under saturated light conditions, sugars and under limited light conditions preferentially assimilate carbon in cal composition. Microalgae cells cultivated photoautotrophically tions.

between 2.0 and 99.69%, indicating the importance of the light supply of light resulted in a reduction in the CO2 fixation rate supply. An analysis of the diagram shows that the discontinuous erence being the experiment carried out with a continuous light 16 h.

These results suggest the possibility of storing light energy by way of exergonic reactions, supplying an excess of energy for later use in subsequent endergonic reactions that can occur in periods in which there is an absence of light. From the operational point of view, this situation would be technologically interesting for use in photobioreactors, in which species capable of storing substantial amounts of light energy would show better performance in the application of this type of process. However, the limited capacity of the majority of microalgae to store light energy, means that in these cases the majority of the energy is dissipated in the reactors in the form of heat [15].

The carbon dioxide fixation rates are associated with the energy received by the cells during the light periods. Maximum carbon dioxide fixation rates of 1.440 g/L/day were found for cultures with a continuous supply of light energy. A linear reduction in the CO2 fixation rates with the reduction in duration of the light period was evident, with the exception of the 12:12 (night:day) cycles. It was also observed that carbon dioxide fixation did not differ significantly (p < 0.05) in cultures grown with dark periods greater than 16 h.

Fig. 3 shows the distribution of the percent carbon dioxide fixed in the biomass under the different conditions evaluated, the reference being the experiment carried out with a continuous light supply. An analysis of the diagram shows that the discontinuous supply of light resulted in a reduction in the CO2 fixation rate between 2.0 and 99.69%, indicating the importance of the light phase of photosynthesis in the subsequent carbon-fixation reactions.

Besides the changes in the rates of biomass production and CO2 fixation, one should also consider that the biomass formed in each photoperiod condition might have a different biochemical composition. Microalgae cells cultivated photoautotrophically under limited light conditions preferentially assimilate carbon in the direction of the synthesis of amino acids and other essential cell constituents, but under saturated light conditions, sugars and starch are formed via the pentose phosphate-reducing pathway [20,21], suggesting the dependence of the biomass composition with the light availability.

So the results obtained for the different light cycles evaluated indicated that the development of technology for the biological fixation of carbon dioxide in photobioreactors depended fundamentally on the access to light energy, which should be provided by solar energy so as to develop clean technology processes. Thus this type of CO2 sequestration process could be very efficient in tropical countries, especially in locations near the equator where the light and dark periods are very similar and are associated with favourable temperature conditions. Martins et al. [22] reported that countries like Brazil show an ample potential for the exploration of solar energy, with mean solar irradiation varying from 4.25 to 6.50 kW/m2 day in the different regions included in its territory. According to these authors, all regions in Brazil receive elevated light energy indices, with the potential for use as an energy source.

4. Conclusions

The development of photobioreactors for carbon dioxide fixation is a potential technology for application in tropical countries with elevated solar light availability. However, in order to predict the real carbon dioxide removal rates and biomass production in such systems, the lack of availability of light energy for part of the time during complete 24-h time periods must be considered, and this was evident from the cultivation of the cyanobacterium Aphanothece microscopica Nägeli under different photoperiod conditions.

Maximum values of 0.770 gbiomass/L.day, 5.100 gbiomass/L and 1.440 gCO2/L day were obtained for volumetric productivity, maximum cell concentration and carbon dioxide fixation rate, respectively, under continuous light regime.

Linear reduction of the performance of the microorganism was evidenced in function of the duration of light regime. Exception to this behaviour was obtained in light cycles of the 12:12 (dark/light), suggesting the importance of the pre-adaptation of the microalgae cultures.

Thus the results obtained in the present study suggest the potential of applying this type of process to remove carbon dioxide. Nevertheless, the duration of the light cycles (night:day) is one fundamental criteria which should therefore be considered when projecting and analysing photobioreactors for the sequestering of carbon dioxide and biomass production.

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Appendix A. Nomenclature

<table>
<thead>
<tr>
<th>C</th>
<th>Percent carbon in the biomass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M&lt;sub&gt;C&lt;/sub&gt;</td>
<td>molecular weight of carbon</td>
</tr>
<tr>
<td>M&lt;sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;&lt;/sub&gt;</td>
<td>molecular weight of CO&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>P&lt;sub&gt;X&lt;/sub&gt;</td>
<td>volumetric growth rate (g/L day)</td>
</tr>
<tr>
<td>R&lt;sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;&lt;/sub&gt;</td>
<td>carbon dioxide fixation rate (g/L day)</td>
</tr>
<tr>
<td>t&lt;sub&gt;0&lt;/sub&gt;</td>
<td>cell residence time in t = 0 (h)</td>
</tr>
<tr>
<td>t&lt;sub&gt;1&lt;/sub&gt;</td>
<td>cell residence time in t = n (h)</td>
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<td>cell concentration in t = 0 (g/L)</td>
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<td>cell concentration in t = n (g/L)</td>
</tr>
<tr>
<td>X&lt;sub&gt;max&lt;/sub&gt;</td>
<td>maximum cell density (g/L)</td>
</tr>
</tbody>
</table>

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