Short Communication

Vertical tubular photobioreactor for semicontinuous culture of *Cyanobium* sp.

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ABSTRACT

We evaluated the kinetic culture characteristics of the microalgae *Cyanobium* sp. grown in vertical tubular photobioreactor in semicontinuous mode. Cultivation was carried out in vertical tubular photobioreactor for 2 L, in 57 d, at 30 °C, 3200 Lux, and 12 h light/dark photoperiod. The maximum specific growth rate was found as $0.127 \, d^{-1}$, when the culture had blend concentration of $1.0 \, g \, L^{-1}$, renewal rate of 50%, and sodium bicarbonate concentration of $1.0 \, g \, L^{-1}$. The maximum values of productivity ($0.071 \, g \, L^{-1} \, d^{-1}$) and number of cycles (10) were observed in blend concentration of $1.0 \, g \, L^{-1}$, renewal rate of 30%, and bicarbonate concentration of $1.0 \, g \, L^{-1}$. The results showed the potential of semicontinuous cultivation of *Cyanobium* sp. in closed tubular bioreactor, combining factors such as blend concentration, renewal rate, and sodium bicarbonate concentration.

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1. Introduction

Microalgal biotechnology has been explored by many researchers because of its potential to enrich foods as nutritional supplement, biofixation of carbon dioxide (Morais and Costa, 2007; Ugwu et al., 2008), generation of biofuels (Costa and Morais, 2011) and extraction of specific biocompounds (Silveira et al., 2007). Growth conditions such as temperature, light, pH, photobioreactor type, operation, addition the microalgae biomass in the nanofibers development (Morais et al., 2010) as well as availability and sources of nutrients directly influence the achievement of biocompounds and the kinetic characteristics of the culture.

Microalgae have been used for centuries as food sources of protein by indigenous tribes in Chad and the Aztecs, who dried them in order to ingest them (Henrikson, 1994). Conventional methods of microalgae cultivation are held in open raceway-type tanks, which use natural or artificial light. However, such a method requires large areas and it may be difficult to control the growing conditions, evaporation, and reduction of light intensity with increasing height of the medium.

Although a good number of photobioreactors have been proposed, only a few of them can be practically used for mass production of algae. One of the major factors that limit their practical application in algal mass cultures is mass transfer (Ugwu et al., 2008). The use of vertical tubular photobioreactor provides greater contact between the light and the culture, increasing the remaining time of gas in the culture medium and therefore the efficiency of nutrients use. In addition, the carbon source is one of the most

costly components of the medium, and its optimal concentration in the culture must be determined, as its excess or limitation affects microalgal growth (Stewart and Hessami, 2005).

Most of the times, large-scale cultivation of microalgae is not economically viable to be performed in batch mode, due to the time required for loading, unloading, and cleaning the photobioreactor. This study aimed to determine the kinetic culture characteristics of the microalgae *Cyanobium* sp. grown in vertical tubular photobioreactor in semicontinuous mode.

2. Experimental section

2.1. Microorganism and culture medium

In this study, *Cyanobium* sp. microalgae were used. For preparing and maintaining the inoculum, the culture medium contained BG11 (Rippka et al., 1979). The concentration of $NaHCO_3$ in the culture medium ranged between 0.4, 1.0 and $1.6 \, g \, L^{-1}$.

2.2. Culture conditions

Cyanobium sp. was cultured in 2 L vertical tubular photobioreactor (Morais and Costa, 2007) with a volume of 1.8 L and initial concentration of $0.40 \, g \, L^{-1}$.

The agitation of crops was carried out by injection of compressed air at a flow rate of 0.3 vvm (Morais and Costa, 2007). The light intensity was of $41.6 \, \mu mol \, m^{-2} \, s^{-1}$ provided by fluorescent day-light type lamps (40 Watts). The experimental apparatus was maintained for 57 d at 30 °C and with 12 h light/dark photoperiod (control with a timer) (Reichert et al., 2006).

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2.3. Analytical determinations

The samples were collected daily for determination of cell concentration, measured by optical density at 670 nm on a spectrophotometer VARIAN Cary 100 model (Costa et al., 2003), with a calibration curve relating optical density with dry weight biomass of *Cyanobium* sp.

2.4. Responses studied

When the *Cyanobium* sp. biomass concentration in the culture reached a predetermined level (0.8, 1.0, and 1.2 g L^{-1}, named as the 'blend concentration') a portion of the medium (30%, 40% or 50% (v/v), the 'renewal rate') was withdrawn and the same amount of fresh medium added. The specific growth rate (μ_x) in the exponential phase was obtained by exponential regression and the biomass doubling time (t_d) calculated using $t_d = (\ln 2)/\mu_x$. Productivity (P_x, g L^{-1} d^{-1}) was obtained according to the equation $P = (X_0 - X_t) \times t^{-1}$, where X_t is the cellular concentration (g L^{-1}) at time t (d) and X_0 (g L^{-1}) the cellular concentration at time t_0 (d). The *Cyanobium* sp. biomass productivity (P_x) was expressed as g L^{-1} d^{-1}.

2.5. Experimental design

A factorial design, Box-Behnken type (Radmann et al., 2007), was proposed and modified with three study factors, each of them ranging at three levels. The change in design was done in the blend concentration, where the central level was replaced by the higher one and vice versa. Table 1 shows the matrix of coded variables of the factorial design and the respective levels of variables.

As the cell concentration reached 0.8, 1.0, or 1.2 g L^{-1} (blend concentration), according to the design matrix, 30%, 40%, and 50% of the culture medium was released, and the same amount of new medium was added. Each experiment was performed for 57 d.

The specific growth rate was obtained by exponential regression in the logarithmic phase of multiplication in each time interval corresponding to their growth cycle (blend concentration). Biomass productivity, defined as the cell mass formed in a given volume per time unit, was measured separately for each blend concentration.

2.6. Statistical analysis

The results from the experimental data were evaluated by comparing the growth curves and the analysis of variance (ANOVA), with a confidence level of 90% ($p \leqslant 0.10$).

Table 1
Specific growth rate (μ_x, d^{-1}), generation time (t_g, d), growth cycles (N), productivity (P_x, g L^{-1} d^{-1}), in the study performed according to the matrix of the factorial design Box-Behnken applied to the culture study of the microalgae *Cyanobium* sp.

Exp.	X_1	X_2	X_3	μ_x	t_g	N	P_x
1	−1	−1	0	0.109	6.33	5	0.036
2	0	−1	0	0.096	7.23	10	0.071
3	−1	+1	0	0.111	6.22	7	0.060
4	0	+1	0	0.127	5.44	6	0.069
5	−1	0	−1	0.049	14.20	6	0.026
6	0	0	−1	0.070	9.93	8	0.046
7	−1	0	+1	0.051	13.70	4	0.018
8	0	0	+1	0.047	14.80	5	0.025
9	+1	−1	−1	0.051	13.53	6	0.070
10	+1	+1	−1	0.056	12.39	5	0.057
11	+1	−1	+1	0.052	13.21	8	0.066
12	+1	+1	+1	0.075	9.29	6	0.034
13	+1	0	0	0.079	8.75	8	0.053
14	+1	0	0	0.072	9.63	7	0.056
15	+1	0	0	0.092	7.54	8	0.061

X_1: Blend concentration, X_2: Renewal rate, X_3: Bicarbonate sodium concentration.

3. Results and discussion

The maximum specific growth rate was 0.127 d^{-1}, in the experiment 4, with blend concentration of 1.0 g L^{-1}, 50% renewal rate, and bicarbonate concentration in the environment of 1.0 g L^{-1}. Radmann et al. (2007) found 0.138 d^{-1} when cultured the microalgae *Spirulina* with blend concentration of 0.4 g L^{-1}, renewal rate of 40%, and 20% of Zarrouk medium diluted in distilled water. Moreover, increasing the blend concentration to 0.6 g L^{-1} and the Zarrouk concentration to 50%, and decreasing the renewal rate to 20% reduced the specific growth rate to 0.038 d^{-1}.

Spirulina cultures performed in the open raceway-type photobioreactor, containing water from the Magueira lagoon supplemented with sodium bicarbonate and urea, had maximum specific growth rate of 0.157 d^{-1}, in the exponential growth stage for 15 days (Costa et al., 2003). However, in experiments with tubular photobioreactor, the microalgae *Cyanobium* sp. had specific growth rate of 0.127 d^{-1}, which was kept for 55 days of culture.

The photobioreactor configuration is important to control the biomass productivity of photosynthetic cultures. Morais and Costa (2007) noted that vertical tubular photobioreactors have better kinetic responses than Erlenmeyer ones for cultures of *Spirulina* sp., *Scenedesmus obliquus*, *Chlorella kessleri*, and *Chlorella vulgaris*.

According to Henrikson (1994), microalgae can double their biomass in less than 7 days. In this study, the shortest generation time was of 5.44 days (experiment 4, blend concentration of 1.0 g L^{-1}, renewal rate of 50%, and bicarbonate concentration in the medium of 1.0 g L^{-1}), proving the advantage of the semicontinuous culture. Such generation time agrees with the shortest time found by Radmann et al. (2007) which was of 5.2 days in semicontinuous culture with *Spirulina platensis*. The generation time decreases as the cell duplication rate increases, economically enabling the cultivation. Microalgae culture is advantageous because their biomass can be doubled in less than one week, while the biomass of terrestrial plants and the concentration of animal protein may take months and years to double (Henrikson, 1994).

The maximum productivity and growth cycles were 0.071 g L^{-1} d^{-1} and 10 cycles (Fig. 1), respectively, in experiment 2 (blend concentration of 1.0 g L^{-1}, renewal rate of 30%, and bicarbonate concentration in the medium of 1.0 g L^{-1}), which also had the longest time of exponential growth stage (55 days). The experiments 6, 11, 13, and 15 had 8 growth cycles along the culture. Reichert et al. (2006) found similar results in semicontinuous cultures in Erlenmeyer with *Spirulina platensis*, blend concentration of 0.5 g L^{-1}, and renewal rate of 25%, during 71 days, in which productivity and growth speed started to decrease after 50 days of culture. Fábregas et al. (2001) performed a semicontinuous culture study with the microalgae *Haematococcus pluvialis* in closed photobioreactors and found maximum productivity with renewal rate of 20%.

In this experiments the *Cyanobium* sp. had accumulation of biomass followed by cell death when exposed to initial cell concentration of 0.10, 0.20 and 0.30 g L^{-1} (absent data), which was fixed with the adoption of the initial concentration of 0.40 g L^{-1}. The photoinhibition is a phenomenon occurring in microalgae culture due to the excess of light; it can lead to alterations that inhibit or slow the photosynthesis by the microalgae, especially because of the accumulation of H_2O_2. Such a phenomenon may be enabled by stress conditions, such as the low cell concentration in the culture, which allows high light incidence on the cells (Papáček et al., 2010; Soleto et al., 2008; Vonshak, 1997). The emitted light flow may cause the photoinhibition depending on the genus and species of the microalgae. *Spirulina* culture generally has optimum growth in conditions of initial cell concentration of 0.15 g L^{-1}.

In this study, the maximum values of specific growth rate, productivity, and amount of growth cycles were observed for the blend concentration of 1.0 g L^{-1}, which had the less biomass
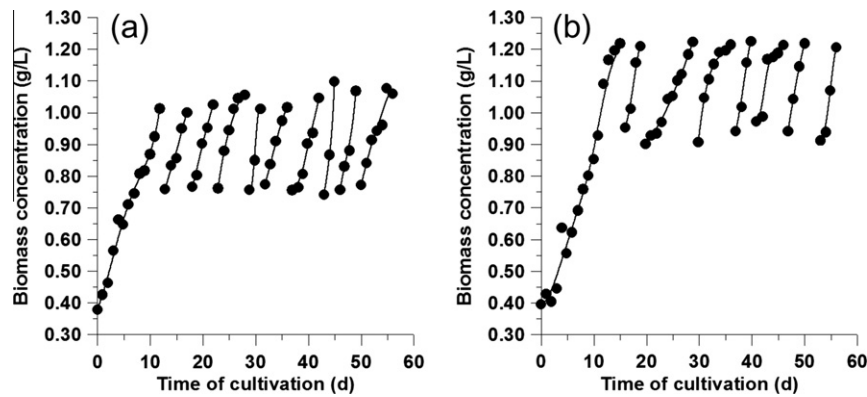
Fig. 1. Cell concentration curve of semicontinuous cultures according to time: (a) blend concentration of 1.0 g L^{-1}, renewal rate of the medium of 30%, and sodium bicarbonate concentration of 1.0 g L^{-1} (experiment 2), (b) blend concentration of 1.2 g L^{-1}, renewal rate of the medium of 40%, and sodium bicarbonate concentration of 1.0 g L^{-1} (experiment 15).

concentration of 0.5 g L^{-1} after the renewal. According to Travieso et al. (2001) the ideal cell concentration for the maximum microalgal productivity is between 0.5 and 0.7 g L^{-1} d^{-1}, in discontinuous processes. In this concentration interval, cultures also are less prone to contamination by other microalgae or microorganisms.

The resulting effects and significance levels of the variance analysis for the Box-Behnken factorial design used in the semicontinuous culture are shown in Table 2. The renewal rate of the medium and its bicarbonate concentration had significant quadratic effect ($p < 0.10$). Considering the effect of the renewal rate on the specific growth rate, the latter was affected negatively when the growth rate decreased with the 40% renewal. Therefore, the behavior of the specific growth rate was found as non-linear, with an increased variable central level (1.0 g L^{-1}).

Considering the productivity, the three factors under study had significant influence ($p < 0.10$), except for the renewal rate of the medium in linear form. The quadratic effect of the renewal rate was significant, with a peak between the rates of 40% and 50%.

The highest specific growth and productivity rates are obtained with rate renewal of 30% and 50%, and bicarbonate concentration between 0.4 and 1.6 g L^{-1}. Meanwhile, the lowest specific growth and productivity rates are obtained in maximum and minimum sodium bicarbonate concentrations, and rate renewal of 40%.

The highest number of growth cycles (10 cycles) occurred with the lowest renewal rates of the medium, and sodium bicarbonate concentrations between 0.4 and 1.6 g L^{-1}. The sodium bicarbonate is the nutrient added mostly to the culture medium, corresponding to 60% of the total costs with nutrients. In this study, the use of 1.6 g L^{-1} of sodium bicarbonate affected negatively the microalgal

growth, which suggests the use of lower concentrations of such nutrient.

Smith et al. (1992) studied the growth of the microalgae Skeletonema costanum in different levels of radiation and nitrogen concentration, and observed that the microorganisms require more energy to incorporate and reduce the nitrate in the medium as the nutrients concentration is elevated, decreasing the photosynthetic efficiency. Such results accord to the ones obtained in this study, because lower productivity and specific growth rates were found with the maximum level of sodium bicarbonate in the medium.

Travieso et al. (2001) studied different renewal rates of the medium during the semicontinuous cultivation of Spirulina platensis in tubular photobioreactor with daily cycles, and observed that the productivity increased according to the renewal rates (5–20%), besides the fact that it decreased from 25% on. The results accord partially to the ones found in this study, as the lower productivity was obtained when the microalgae was cultured with renewal rate of 40% and sodium bicarbonate concentrations of 0.4 and 1.6 g L^{-1}. However, a three times higher productivity was obtained with renewal rates of 30% and 50% associated with sodium bicarbonate concentration of 1.0 g L^{-1}.

4. Conclusions

The microalgae Cyanobium sp. had the best kinetic responses with bicarbonate concentration of 1.0 g L^{-1}, blend concentration of 1.0 g L^{-1}, and renewal rate of the medium of 30% or 50%. Under such conditions, the maximum values of specific growth rates, productivity, and growth cycles were 0.127 d^{-1}, 0.071 g L^{-1} d^{-1}, and 10, respectively.

The combination of blend concentration, renewal rate of the medium, and sodium bicarbonate concentration in a way to reach the highest productivity and growth specific rates, as well as the support of the microorganism in exponential growth stage, during 55 days.

Table 2
Statistical significance (p) and effects obtained from the factorial design analysis used in the semicontinuous culture of the Cyanobium sp.

Factor	μ_x (d^{-1})		P_x (g L^{-1} d^{-1})	
	p	Effect	p	Effect
X_1 (L)	0.2210	−0.0112 ± 0.0080	0.0007[a]	0.0217 ± 0.0029
X_1 (Q)	0.2187	0.0106 ± 0.0075	0.0550[a]	0.0068 ± 0.0027
X_2 (L)	0.1438	0.0163 ± 0.0094	1.0000	0.0056 ± 0.0034
X_2 (Q)	0.0585[a]	−0.0162 ± 0.0066	0.0015[a]	−0.0151 ± 0.0024
X_3 (L)	0.7161	−0.0036 ± 0.0094	0.0112[a]	−0.0135 ± 0.0034
X_3 (Q)	0.0021[a]	0.0387 ± 0.0066	0.0016[a]	0.0150 ± 0.0024
$X_1 \times X_2$	0.6942	0.0045 ± 0.0109	0.0022[a]	−0.0230 ± 0.0046
$X_1 \times X_3$	0.6942	0.0045 ± 0.0109	0.6681	−0.0018 ± 0.0039
$X_2 \times X_3$	0.7119	0.0050 ± 0.0127	0.0983[a]	−0.0095 ± 0.0046

X_1: Blend concentration; X_2: Renewal rate of the medium; X_3: Sodium bicarbonate concentration; L: Linear effect; Q: Quadratic effect; μ_X: Specific growth rate; P_X: Productivity.

[a] Statistically significant for a confidence interval of 90%.

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