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The cultivation of microalgae *Cyanobium* sp. and *Chlorella* sp. in different culture media and stirring setting

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Microalgae can serve as a source of biocompound with high nutritional and commercial potential, such as fatty acids, vitamins and pigments. The microalgae composition is influenced by the physical and chemical conditions of the culture medium and environment in which they are grown, and the composition can be manipulated to produce specific biomolecules. The lack of stirring or the employment of stirring inefficient in the microalgae cultivation, limits the development of the culture because may have areas of stagnant culture, in which the cells do not receive light, which affects photosynthesis and cellular growth. The biomass concentration too is directly influenced by concentration of each nutrient culture medium. Hence, the need to add macronutrients and micronutrients in the ideal concentration and correct form as regards mass concentration and absorption availability. The objective of this study was to assess the growth of the microalgae *Cyanobium* sp. and *Chlorella* sp. in different culture media and stirring settings. For this, the cultures were carried out in Erlenmeyer flasks and Raceway type bioreactors, respectively, for 10 days at 30°C, with illumination of 41.6 µmol m^{-2} $s^{-1,}$ and with a 12 h light/dark photoperiod. The maximum biomass concentrations (0.56 and 0.66 g L^{-1}), specific growth rate (0.30 and 0.17 d^{-1}) and productivities (0.12 and 0.09 g L^{-1} d^{-1}), were observed in *Cyanobium* and *Chlorella*, respectively, when they were grown in BG11 medium with 0.40 g L^{-1} $NaHCO_3$. Regarding the stirring, the best responses were obtained when the assays were carried out with stirring by two submersible pumps, with maximum biomass concentrations of 1.21 g L^{-1} for the *Cyanobium* sp. and 0.93 g L^{-1} for the *Chlorella* sp.

Key words: BG11 medium, H/2 medium, ASM medium, Raceway, submersible pump.

INTRODUCTION

Microalgae have been studied in biotechnology research due to their nutritional, economic and ecological importance. These microorganisms can use solar energy efficiently to transform wastewater, CO_2 and additional nutrients into a biomass (Batista et al., 2013). The biomass produced can be exploited as a source of biocompounds with high nutritional and commercial value, some of which are used in the formulation of foods

and feeds and some of which can be used to help minimize problems such as human malnutrition (Toledo-Cervantes et al., 2013; Ho et al., 2011).

Microalgae cultivation is strongly influenced by the setting of stirring and components of the culture medium, since both factors can maintain the maximum absorption of nutrients for the microorganism. According to Borowitzka (1999), microalgae require stirred systems to allow high rates of growth because a good stirring increases the absorption of nutrients and allows better oxygen removal from the medium. In addition, a good stirring promotes amount of light essential, for in the absence of any other limiting factor, the cell concentration increases with light intensity until reaching maximum biomass concentration that is denominated "saturation level" (Bezerra et al., 2012).

The bioreactor employed for microalgae cultivation has direct influence on the kinetic results of this culture. Raceway ponds are the most widely used and economical one for the large scale cultivation. One of the major advantages of open ponds is that they are easier to construct and operate than most closed systems. One disadvantage is the biomass productivity limited due to poor utilization of light and possibility of contamination as compared to the closed bioreactor (photobioreactor) (Vasumathi et al., 2012). The depth of the bioreactor must take into account the need to provide light to the cells and the need to maintain a liquid column suitable for mixing and to avoid large ionic exchange due to evaporation (Borowitzka, 1999). In bioreactors without stirring, the light limits the development of the culture. These bioreactors may have areas of stagnant culture, in which the cells do not receive light, which affects photosynthesis and cellular growth (Pulz, 2001).

The culture medium has a direct influence on cellular growth, as well as on the biomass composition of microalgae (Richmond, 2004). In this context, microalgae, like other microorganisms, require sources of carbon, nitrogen, phosphorus and other micronutrients (Kumar et al., 2010), and the nutrients that make up the medium must be dissolved (available) for biochemical use by the microalgae. This way, the stirring of the bioreactor and the cultivation medium are two important factors to be studied.

The objective of this study was to assess the growth of the microalgae *Cyanobium* sp. and *Chlorella* sp. with different culture media and stirring settings.

MATERIALS AND METHODS

Selection of nutritional conditions

The studied microalgae were *Chlorella* sp. (Henrard et al., 2014) and *Cyanobium* sp. (Henrard et al., 2011). The culture media used to cultivate the *Cyanobium* microalgae were ASM, BG11, BG11+$NaHCO_3$, H/2 and H/2+$NaHCO_3$, while BG11, BG11+$NaHCO_3$, H/2, H/2+$NaHCO_3$, MBM, MBM+$NaHCO_3$, MC and MC+$NaHCO_3$ were used for the *Chlorella* sp.

The ASM medium contained (g L^{-1}): $NaNO_3$ (0.17), $MgSO_4$ (0.049), $MgCl_2$ (0.041), $CaCl_2$ (0.029), K_2HPO_4 (0.0174), Na_2HPO_4 (0.0356); H_3BO_3 (0.00248), $MnCl_2$ (0.00139), $FeCl_3$ (0.00108), $ZnCl_2$ (0.00034), $CoCl_2$ (0.00002), $CuSO_4$ (0.00186), and Na_2EDTA (0.00744) (Gorham et al., 1964). The BG11 medium contained (g L^{-1}): $NaNO_3$ (1.50), $K_2HPO_4{\cdot}3H_2O$ (0.04), $MgSO_4{\cdot}7H_2O$ (0.075), $CaCl{\cdot}2H_2O$ (0.036), $C_6H_{11}FeNO_7$ (0.006), EDTA disodium magnesium (0.001), Na_2CO_3 (0.02), $C_6H_8O_7$ (0.006), H_3BO_3 (0.00286); $MnCl_2{\cdot}4H_2O$ (0.00181) $ZnSO_4{\cdot}7H_2O$ (0.000222), $Na_2MoO_4{\cdot}2H_2O$ (0.00039), $CuSO_4{\cdot}5H_2O$ (0.000079), $Co(NO_3)_2{\cdot}6H_2O$ (0.0000494) (Rippka et al., 1979). The BG11 medium was modified by adding 0.40 g L^{-1} $NaHCO_3$.

The H/2 medium contained (g L^{-1}): $NaNO_3$ (0.075), $NaH_2PO_4{\cdot}H_2O$ (0.005), $FeCl_3{\cdot}6H_2O$ (0.00315), $Na_2EDTA{\cdot}2H_2O$ (0.00436), $CuSO_4{\cdot}5H_2O$ (0.0098), $Na_2MoO_4{\cdot}2H_2O$ (0.0063), $ZnSO_4{\cdot}7H_2O$ (0.022), $CoCl_2{\cdot}6H_2O$ (0.010), $MnCl_2{\cdot}4H_2O$ (0.18), Vitamin B_{12} (0.001), Biotin (0.001), Thiamine (0.20) (Guillard, 1975; Guillard and Ryther, 1962). The H/2 medium was modified by adding 0.40 g L^{-1} $NaHCO_3$. The MBM medium contained (g L^{-1}): KNO_3 (0.25); $CaCl_2$ (0.01); $MgSO_4{\cdot}7H_2O$ (0.075); K_2HPO_4 (0.075); KH_2PO_4 (0.175); NaCl (0.025); $FeSO_4{\cdot}7H_2O$ (0.02), H_3BO_3 (0.00286), $MnCl_2{\cdot}4H_2O$ (0.00181), $ZnSO_4{\cdot}7H_2O$ (0.000222), $CuSO_4{\cdot}5H_2O$ (0.00079) and $NaMoO_4$ (0.000015) (Watanabe, 1960). The MBM medium was modified by adding 0.40 g L^{-1} $NaHCO_3$. The MC medium contained (g L^{-1}): KNO_3 (1.25); $MgSO_4{\cdot}7H_2O$ (1.25); KH_2PO_4 (1.25); $FeSO_4{\cdot}7H_2O$ (0.02), H_3BO_3 (0.00286), $MnCl_2{\cdot}4H_2O$ (0.00181), $ZnSO_4{\cdot}7H_2O$ (0.000222), $CuSO_4{\cdot}5H_2O$ (0.000079) and $NaMoO_4$ (0.000015) (Watanabe, 1960). The MC medium was modified by adding 0.40 g L^{-1} $NaHCO_3$.

The culturing was carried out in duplicate, under controlled conditions in a controlled temperature chamber at 30°C, and the illumination of 41.6 µmol m^{-2} s^{-1} was provided by 40 W daylight-type fluorescent lamps with a 12 h light/dark photoperiod (Reichert et al., 2006) for 10 days. The microalgae were grown in closed Erlenmeyer flask photobioreactors (0.50 L) with a working volume of 0.40 L and continuous stirring by injection of compressed air. The initial concentrations of the cultures of *Cyanobium* sp. and *Chlorella* sp. were 0.10 and 0.20 g L^{-1}, respectively. After selecting the culture medium with the best results, the different stirring settings were studied.

Study of the different stirring settings

To evaluate the different methods of stirring, *Cyanobium* sp. and *Chlorella* sp. were grown in open Raceway type bioreactors (0.70 m long, 0.20 m wide and 0.075 m deep) (Radmann et al., 2007) with 5 L of working volume (Henrard et al., 2014).

The stirring was continuous using eight different configurations: rotating blades at 15 rpm (Figure 1A); rotating blades and air injection through 2 porous curtains (900 mm each), in a spiral, attached to the bottom of the bioreactor (Figure 1B); rotating blades and air injection through 3 elongated porous stones (150 mm each) (Figure 1C); rotating blades and air injection through 6 cylindrical porous stones (30 mm each) (Figure 1D); 2 submersible pumps with a flow of 60 L h^{-1} each (Figure 1E); 1 submersible pump with a flow of 60 L h^{-1} (Figure 1F); 2 porous curtains (600 mm each), attached to the bioreactors with air injection (Figure 1G); 6

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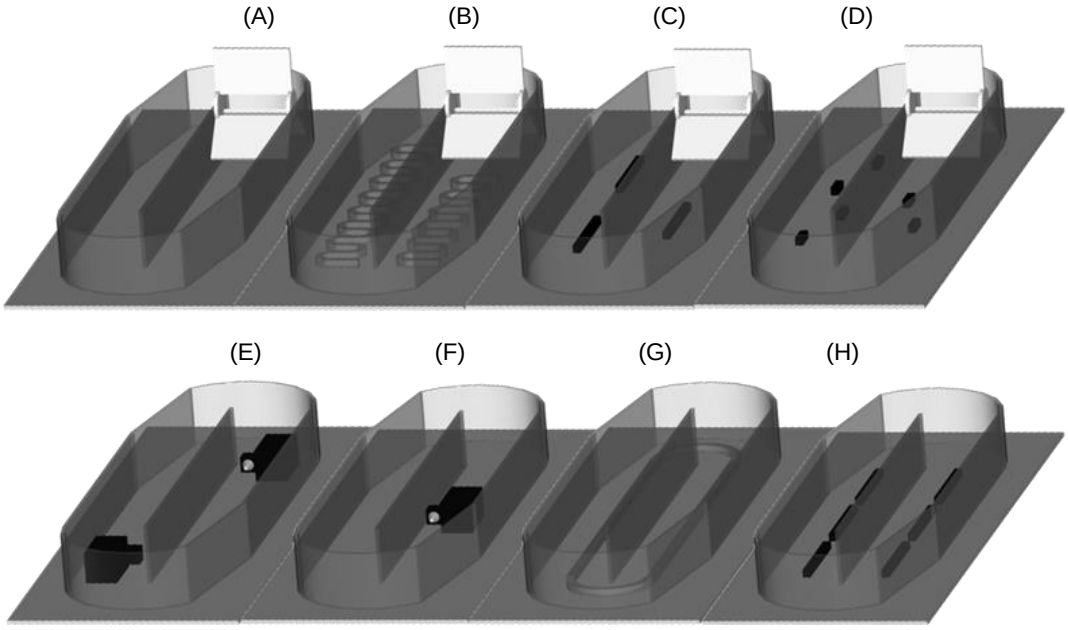

Figure 1. Schematic drawing of each Raceway type bioreactor, with its stirring settings: (A) rotating blades at 15 rpm; (B) rotating blades and porous curtains (900 mm each), in a spiral; (C) rotating blades and 3 elongated porous stones (150 mm each); (D) rotating blades and 6 cylindrical stones (30 mm each); (E) 2 submersible pumps with a flow of 60 L h^{-1} each; 1 submersible pump with a flow of 60 L h^{-1}; (G) 2 porous curtains (600 mm each) and (H) 6 elongated porous stones (150 mm each).

elongated porous stones (150 mm each) with air injection (Figure 1H). Figure 1 presents the bioreactors of the cultures with their respective stirrings.

The culturing was carried out in duplicate, under controlled conditions in a temperature-controlled chamber at 30°C, under illumination of 41.6 µmol m^{-2} s^{-1} provided by 40 W daylight-type fluorescent lamps with a 12 h light/dark photoperiod (Reichert et al., 2006) for 10 days. The initial concentrations of the *Cyanobium* sp. and *Chlorella* sp. cultures were 0.35 and 0.20 g L^{-1}, respectively. The medium culture selected for this stage was the BG11 with addition of 0.4 g L^{-1} of $NaHCO_3$.

Analytical measurements

The samples were collected every 24 h to measure the biomass concentration, calculated using the optical density at 670 nm in a spectrophotometer (FEMTO 700 Plus, São Paulo – SP - Brazil) with a calibration curve based on the association between the optical density and the dry weight of biomass for each strain (Costa et al., 2002). The pH was also monitored every 24 h using a digital pH meter (Quimis Q400H, Diadema - SP - Brazil).

Studied responses

The daily values of biomass concentration were used to calculate the maximum specific growth rates, maximum biomass concentration and maximum biomass productivity. The maximum specific growth rate (μ_{max}, d^{-1}) was calculated by exponential regression of the logarithmic phase of the cellular growth curve.

The maximum biomass concentration (X_{max}, g L^{-1}) is the maximum value of cellular concentration obtained. The biomass productivity (P, g L^{-1} d^{-1}) was obtained according to the equation $P = (X_1 - X_0)(t - t_0)^{-1}$, in which X_1 is the biomass concentration at time t (d) and X_0 (g L^{-1}) is the biomass concentration at time t_0 (d). P_{max} is the maximum value of biomass productivity found during the cultivation.

Statistical analysis

The results obtained from the experimental data for the microalgae were compared using analysis of variance (ANOVA), followed by Tukey's test, with a confidence interval of 95%.

RESULTS AND DISCUSSION

Selection of nutritional conditions

The choice of culture medium is essential for high biomass production through microalgae cultivation. Culture media can be defined based on previous studies with strains of known microalgae. However, if the nutritional requirements of a strain are not known, a trial and error approach is necessary, using different culture media that may promote the growth of a wide range of microalgae (Mutanda et al., 2011; Scott et al., 2010).

The highest biomass concentrations for *Cyanobium* sp. and *Chlorella* sp. (0.56 and 0.66 g L^{-1}, respectively) were obtained in the BG11 culture medium with the addition of 0.40 g L^{-1} of sodium bicarbonate ($p \leq 0.05$). In all of the assays, the adaptation phase (lag) was missing from the growth profile observed in the microalgae (Figures 2 and

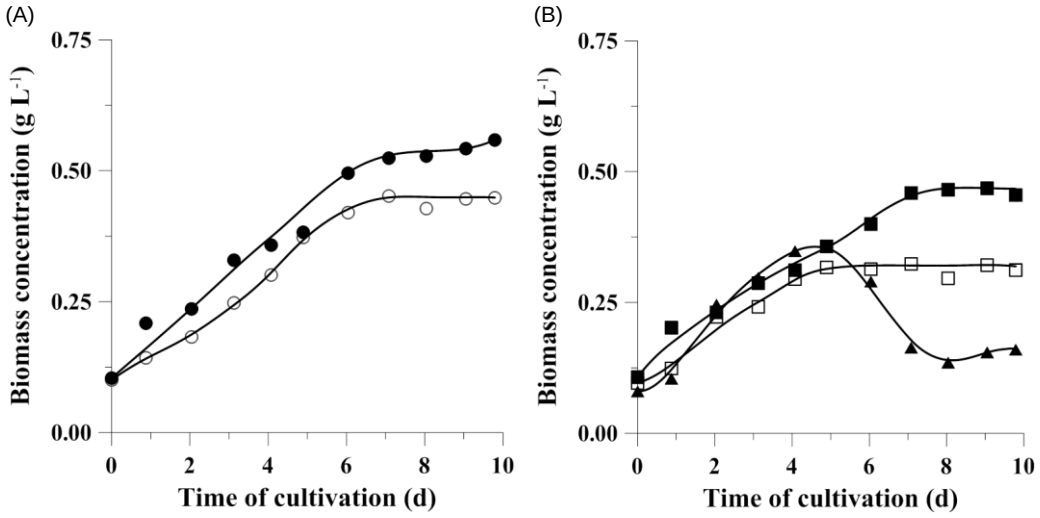

Figure 2. Growth profiles of the assays carried out with different media for *Cyanobium* sp.: (A) BG11 (○), BG11 + 0.40 g L^{-1} $NaHCO_3$ (●); (B) ASM (▲), H/2 (□) e H/2 + 0.40 g L^{-1} $NaHCO_3$ (■).

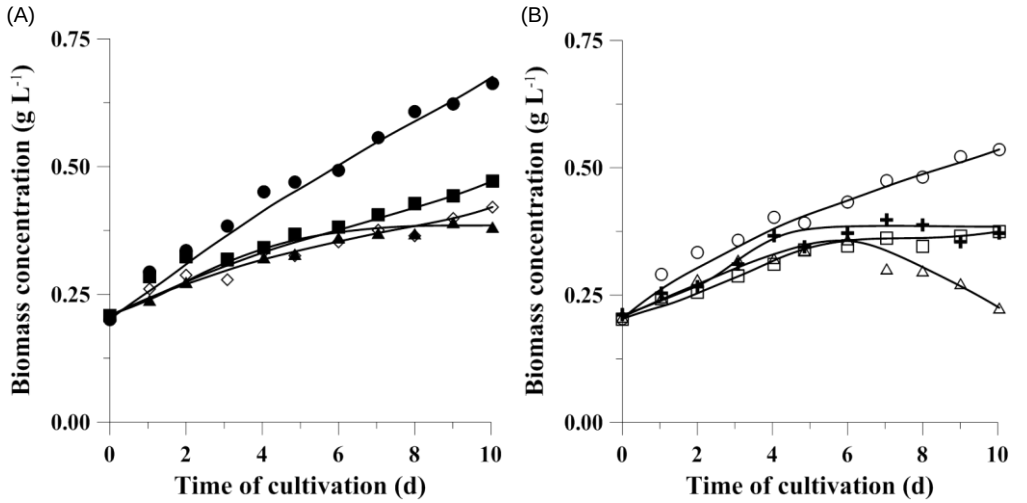

Figure 3. Growth profiles for the assays carried out with different culture media for *Chlorella* sp.: (A) BG11 + 0.40 g L^{-1} $NaHCO_3$ (●), H/2 + 0.40 g L^{-1} $NaHCO_3$ (▲), MBM + 0.40 g L^{-1} $NaHCO_3$ (■) and MC + 0.40 g L^{-1} $NaHCO_3$ (◊); (B) BG11 (○), H/2 (Δ), MBM (□) and MC (**+**).

3), despite the fact that the microalgae were not adapted before the assays.

The assays were 10 days long and the *Cyanobium* sp. showed cellular growth until the 7th day of cultivation with standard BG11, BG11 + $NaHCO_3$ (Figure 2a) and H/2 + $NaHCO_3$ (Figure 2b). When grown in ASM medium, the growth phase continued until the 5th day, reaching a maximum biomass concentration of 0.35 g L^{-1}, followed by cellular death (Figure 2B). For these two nutritional conditions (ASM medium and H/2 medium without $NaHCO_3$), the biomass concentrations of *Cyanobium* sp. were statistically equal ($p = 0.18$) (Table 1).

Chlorella sp. presented cellular growth during the entire 10 days of culture when maintained in BG11 medium, with and without bicarbonate, and in MBM with sodium bicarbonate (Figure 3A and B). Of these nutritional conditions, the biomass concentration was superior ($p < 0.05$) in the cultures grown in standard BG11 medium (Table 1).

The standard H/2 medium led to a cell death phase in *Chlorella* sp. after six days of culture (Figure 3B), leading to a lower maximum biomass concentration ($p < 0.05$) than in *Cyanobium* grown in H/2 medium with sodium bicarbonate. This attenuated growth may have occurred because of the limitation or depletion of nutrients essential for cellular development (such as carbon) because

Table 1. Maximum biomass productivity (P_{max}), maximum biomass concentration (X_{max}) and maximum specific growth rate (μ_{max}) for *Cyanobium* sp. and *Chlorella* sp., when grown in different culture media (ASM, BG11, H/2, MBM and MC).

Culture medium	P_{max} ($g\ L^{-1}\ d^{-1}$)	X_{max} ($g\ L^{-1}$)	μ_{max} (d^{-1})
***Cyanobium* sp.**			
ASM	0.08^{a}	0.35^{a}	0.22^{a}
BG11	0.05^{b}	0.45^{b}	0.25^{b}
BG11 + $NaHCO_3$	0.12^{c}	0.56^{c}	0.30^{c}
H/2	0.06^{d}	0.32^{a}	0.28^{d}
H/2 + $NaHCO_3$	0.11^{e}	0.46^{b}	0.18^{e}
***Chlorella* sp.**			
BG11	0.08^{a}	0.53^{a}	0.09^{a}
BG11 + $NaHCO_3$	0.09^{b}	0.66^{b}	0.17^{b}
H/2	0.04^{cd}	0.36^{c}	0.09^{a}
H/2 + $NaHCO_3$	0.03^{c}	0.39^{c}	0.09^{a}
MBM	0.04^{ce}	0.37^{c}	0.10^{c}
MBM + $NaHCO_3$	0.07^{f}	0.47^{d}	0.07^{d}
MC	0.04^{de}	0.40^{c}	0.12^{e}
MC + $NaHCO_3$	0.05^{g}	0.42^{cd}	0.08^{f}

Superscript letters equal within each column refer to statistically equal means ($p > 0.05$).

the composition of this culture medium has a low nutrient concentration.

The nutrients that are present in the culture medium must be provided in sufficient quantities so that the algal growth rate is maintained at peak levels (Vasumathi et al., 2012). Carbon is the nutrient required at high concentrations for microalgae. This demand is high because the carbon forms carbon skeletons for the subsequent synthesis (Grobbelaar, 2004) of proteins, lipids and carbohydrates within cells. The origin of the total inorganic carbon dissolved in microalgal cultures is HCO_3^-, the predominant species. The concentration of HCO_3^- in the medium determines the rate of formation of CO_2. If there is a low concentration of HCO_3^- in the medium, the growth of microalgae may be limited by the lack of this nutrient (Richmond, 2004)

The results of this study are consistent regarding the addition of sodium bicarbonate because the cell concentration of the cultures with both species of microalgae was increased by adding this inorganic salt in the medium. According to Hughes and Benemann (1997), one alternative to minimize production costs, is to substitute the carbon source in the growth medium with carbon dioxide that originates from the burning of fossil fuels, for the photosynthetic and autotrophic growth of microalgae.

According to Huang et al. (2009), in addition to nutrients such as nitrogen, phosphorus, potassium and carbon, trace elements are also important for the growth of autotrophic and heterotrophic microorganisms. The results obtained in this study demonstrate the influence of the composition of the culture medium that was used, in line with Huang et al. (2009).

When the microalgae *Cyanobium* and *Chlorella* were grown with ASM and H/2 media, respectively, both of them presented cellular growth until the 5th day of cultivation; however, at the 6th day, they began to enter a cellular decline phase because these culture media are usually used for maintenance of the inoculum. The nutrients made up small amounts of the total volume ($mL_{nutrient}\ L_{medium}^{-1}$), and their composition did not include a carbon source, which is a key nutrient for the cellular growth of microalgae.

The better maximum specific growth rates (0.30 and 0.17 d^{-1}) and maximum biomass productivity (0.12 and 0.09 $g\ L^{-1}\ d^{-1}$) for *Cyanobium* sp. and *Chlorella* sp., respectively, were obtained when the microalgae were grown in BG11 medium with sodium bicarbonate (Table 1). Comparing the initial concentrations of the experiments for *Cyanobium* (0.10 $g\ L^{-1}$) and for *Chlorella* (0.20 $g\ L^{-1}$) shows that the biomass productivity and specific growth rates were higher ($p \leq 0.05$) when the initial biomass concentration of the culture was 0.10 $g\ L^{-1}$. Maintaining high productivity with increasing biomass concentration is important from an economic standpoint and is one of the challenges of growing microalgae. Increased cell density adversely affected the incidence of light and, in discontinuous cultivation; this implies a drop in the osmotic pressure of the medium, due to the consumption of nutrients. Both factors have a potential effect on the productivity and growth rate (Vonshak et al., 1982).

Nitrogen is the second most important nutrient and varies from 1 to 10% in the composition of microalgae (Grobbelaar, 2004). A low concentration of nitrogen in the culture medium encourages the accumulation of lipids and polysaccharides in the cells. With an abundant supply of nitrogen, the cultures tend to produce increasing concentrations of proteins and chlorophyll in the cells. When there is a low concentration of nitrogen available for the microalgae, the rate of cellular growth is reduced (Lourenço et al., 2004).

The ASM, BG11, H/2, MBM and MC media contain 0.17 $g\ L^{-1}$ $NaNO_3$; 1.50 $g\ L^{-1}$ KNO_3; 0.075 $g\ L^{-1}$ $NaNO_3$; 0.25 $g\ L^{-1}$ KNO_3 and 1.25 $g\ L^{-1}$ KNO_3, respectively. The largest biomass concentrations were obtained in the BG11 and MC media, which contained maximum amounts of nitrate. These values are in agreement with Grobbelaar (2007), who reported that one of the most important factors to achieve high biomass productivity and concentration is the nutritional content of the medium. The study of the influence of nutritional parameters for the growth of microalgae is of great importance because the use of certain nutrients in the culture medium can increase production costs and

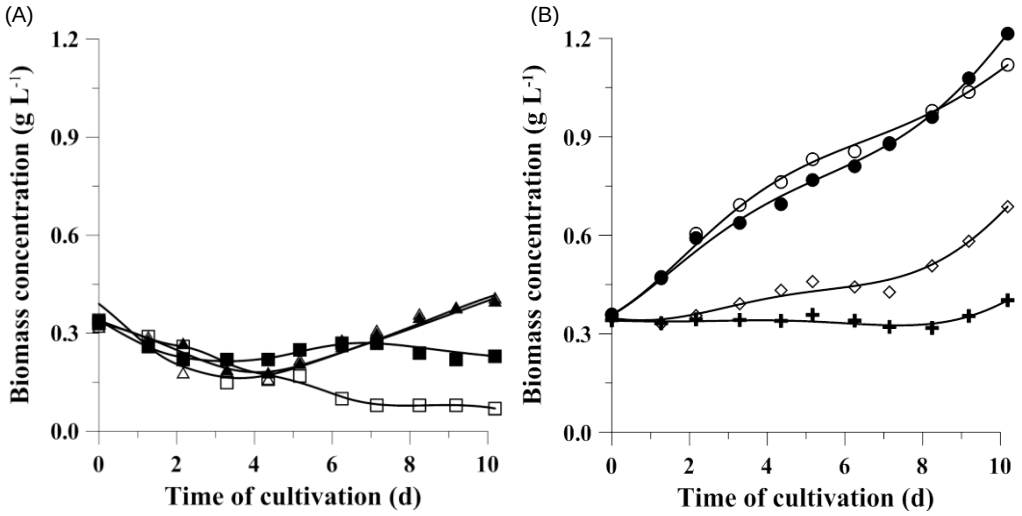

Figure 4. Growth profiles for *Cyanobium sp*. cultivated with the following stirring settings (A): rotating blades (■), rotating blades and porous curtain (□), rotating blades and elongated stones (Δ), rotating blades and cylindrical stones (▲); (B): 2 submersible pumps (●), 1 submersible pump (○), porous curtain (◊) and elongated stones (+).

Table 2. Maximum biomass productivity (P_{max}), maximum biomass concentration (X_{max}) and maximum specific growth rate (μ_{max}) for *Cyanobium* sp. and *Chlorella* sp., when grown in different stirring settings.

Stirring settings	*Cyanobium* sp.			*Chlorella* sp.		
	X_{max} (g L^{-1})	P_{max} (g L^{-1} d^{-1})	μ_{max} (d^{-1})	X_{max} (g L^{-1})	P_{max} (g L^{-1} d^{-1})	μ_{max} (d^{-1})
Rotating blades	-	-	-	0.48^{a}	0.064^{a}	0.071^{a}
Rotating blades and porous curtain	-	-	-	$0.45^{a,b}$	0.048^{b}	0.086^{b}
Rotating blades and elongated stones	0.41^{a}	0.008^{a}	0.150^{a}	$0.44^{a,b}$	0.082^{c}	0.068^{a}
Rotating blades and cylindrical stones	0.40^{a}	0.007^{a}	0.140^{a}	0.43^{b}	0.029^{d}	$0.092^{b,c}$
2 submersible pumps	1.21^{b}	0.108^{b}	0.183^{b}	0.93^{c}	0.111^{e}	0.263^{d}
1 submersible pump	1.12^{c}	0.113^{c}	0.176^{b}	0.71^{d}	0.067^{f}	0.133^{e}
Porous curtains	0.68^{d}	0.034^{d}	*N.E.P	0.29^{e}	0.016^{g}	0.041^{f}
Elongated stones	0.41^{a}	0.008^{a}	*N.E.P	0.24^{f}	0.014^{h}	0.022^{g}

Superscript letters equal within each column refer to statistically equal means ($p > 0.05$); *N.E.F: No exponential phase of growth.

influence the growth and/or composition of the biomass (Sassano et al., 2007).

Study of the different stirring settings

In the cultures with *Cyanobium* sp. (Figure 4A), only the assays with submerged pumps had no adaptation phase (lag), and presented cellular growth during the 10 days of cultivation. In the assay carried out with stirring only by rotating blades, the cultivation had remained constant with no cellular growth or decline. In the experiment with rotating blades and porous curtains, a cellular decline was observed. Insufficient stirring may influence the development of cells, leading them to a regimen of low light incidence, which impairs their growth (Hosaka et al., 1995). The retention of biomass in the porous curtain probably hinders the aeration and homogenization of the culture, and prevents cells from receiving enough light for photosynthesis and growth.

In the assays with *Cyanobium* sp. (Figure 4B), which was carried out with aeration and elongated porous stones, no decline of biomass concentration was observed. However, biomass precipitation occurred in the bioreactor, which slowed growth, and this differed from the experiments that were stirred with 1 or 2 submersible pumps and porous curtains. The cultures that used a porous curtain presented a biomass growth of 0.68 g L^{-1} (Table 2), but precipitation of the biomass was also detected, indicating that the stirring was insufficient to

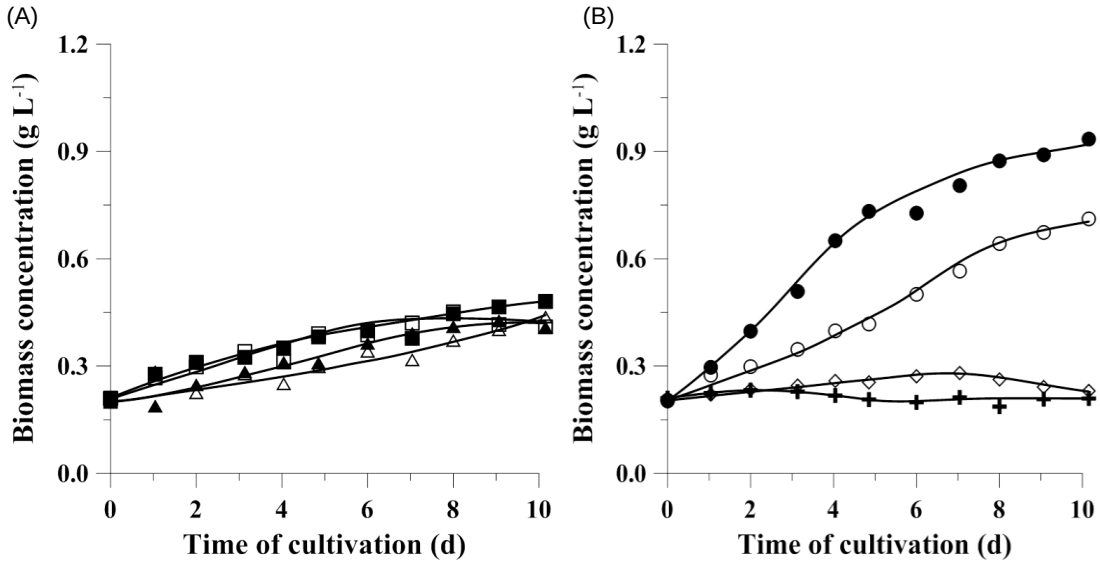

Figure 5. Growth profiles for *Chlorella sp*. cultivated with the following stirring settings: (A): rotating blades (■), rotating blades and porous curtain (□), rotating blades and elongated stones (Δ), rotating blades and cylindrical stones (▲); (B): 2 submersible pumps (●), 1 submersible pump (○), porous curtain (◊) and elongated stones (+).

maintain homogenization of the cultures. These data are consistent with those of Vonshak (1997), who stated that when high luminosity is present, photoinhibition may occur, which is the loss of photosynthetic capacity due to damages caused by light intensities above the level required for photosynthesis. On the other hand, in bioreactors with insufficient stirring, the development of the culture is limited by a lack of luminosity (Borowitzka, 1999).

The maximum biomass concentrations of the *Cyanobium* cultures with 1 or 2 submersible pumps were significantly different ($p \leq 0.05$): 1.12 and 1.21 g L^{-1} (Table 2), respectively. The maximum specific growth rates (0.17 and 0.18 d^{-1}) (Table 2) for *Cyanobium* sp. were obtained with 1 and 2 submersible pumps, respectively, with no significant difference ($p > 0.05$). The maximum biomass productivity (0.11 g L^{-1} d^{-1}) was observed when the microalgae was grown with just 1 submersible pump, which was statistically different ($p \leq 0.05$) from the other stirring settings (Table 2). The stirring of the microalgae cultures is related to specific factors that influence cellular growth. The stirring prevents the formation of cellular clusters and ensures enough light incidence for the cells (Grima et al., 1996), enabling the uptake of atmospheric CO_2 and liberation of O_2 from the interior of the liquid medium. Furthermore, stirring homogenizes the levels of nutrients and gases in the medium (Jiménez et al., 2003).

In cultures carried out with stirring by air injection through elongated porous stones and porous curtain (Figure 5B), the microalgae *Chlorella* sp. presented no cellular growth during the 10 days of cultivation. When *Chlorella* sp. was stirred by rotating blades, the biomass concentration has achieved 0.48 g L^{-1}. The microalga grew throughout the entire cultivation but did not differ from the cultures with stirring using rotating blades with porous curtains and rotating blades with elongated stones (Figure 5A).

The greater the intensity of light to which cells are exposed during the day, the greater the rate of respiration in the dark period, and this respiration can consume up to 35% of the biomass produced in the light period (Torzillo et al., 1991). Therefore, the nocturnal loss of biomass in non-dense cultures that receive high light intensities (as occurs at the beginning of the cultures), contributes to keeping the cell density at a low level, and growth is slow during this period. Likely, when the *Chlorella* was stirred by just rotating blades or compressed air through elongated stones and porous curtains, it did not present formation of biomass during the entire culture due to a lack of stirring, which led to low productivities and possible consumption of part of the biomass produced during the light phase.

The higher responses for *Chlorella* sp. were obtained from stirring with 1 and 2 submersible pumps (Figure 5B), which generated significantly different ($p \leq 0.05$) maximum biomass concentrations of 0.71 and 0.93 g L^{-1}, maximum specific growth rates of 0.13 and 0.26 d^{-1} and maximum biomass productivities of 0.07 and 0.11 g L^{-1} d^{-1}, respectively. Several factors influence the productivity, including the inefficient conversion of light into biomass, accumulation of oxygen in the culture, biomass consumption during respiration, the presence of nutrients in the culture medium and photoinhibition (Stewart and

Hessami, 2005). The maximum biomass concentrations are important for obtaining cell densities that are high enough to make it economically viable to produce microalgae cultures with high operating costs. This way, the results of this study demonstrate that the highest biomass concentrations for *Cyanobium* and *Chlorella* were obtained when they were cultivated with submersible pumps: an increase of 95% in biomass productivity was obtained in these cultures as compared to those stirred with porous curtains and rotating blades with elongated stones.

Conclusions

Cyanobium sp. and *Chlorella* sp. has achieved maximum biomass concentrations (0.56 and 0.66 g L^{-1}, respectively), maximum biomass productivity (0.12 and 0.09 g L^{-1} d^{-1}) and the maximum specific growth rates (0.30 and 0.17 d^{-1}) in BG11 medium supplemented with 0.40 g L^{-1} of sodium bicarbonate. Thus, the medium selected for the studied with different stirring settings was the BG11 with addition of $NaHCO_3$. In this study, *Cyanobium* sp. and *Chlorella* sp. had achieved maximum biomass concentrations (1.21 and 0.93 g L^{-1}, respectively) and maximum specific growth rates (0.18 and 0.27 d^{-1}, respectively) when stirred with two submersible pumps. The maximum biomass productivity of 0.11 g L^{-1} d^{-1} was achieved for *Cyanobium* sp. (with one submersible pump) and *Chlorella* sp. (with two submersible pumps). These results show that the proper selection of nutrients for microalgae and the continuous stirring of the culture improve the efficiency of biomass production, providing light energy and nutritional conditions for cellular multiplication during the entire cultivation period.

Conflict of interests

The authors did not declare any conflict of interest.

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