academicJournals

Vol. 10(21), pp. 768-774, 7 June, 2016
DOI: 10.5897/AJMR2015.7641
Article Number: 8BAC5B458769
ISSN 1996-0808
Copyright © 2016
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Biofixation of CO_2 on a pilot scale: Scaling of the process for industrial application

Felipe Camerini, Michele da Rosa Andrade Zimmermann de Souza, Michele Greque de Morais, Bruna da Silva Vaz, Etiele Greque de Morais and Jorge Alberto Vieira Costa*

Laboratory of Biochemical Engineering, College of Chemical and Food Engineering, Federal University of Rio Grande, Rio Grande, RS, Brazil.

Received 23 June, 2015; Accepted 18 February, 2016

The use of *Spirulina* in CO_2 biofixation, aside from its contribution to reducing the greenhouse effect, enables the use of the biomass to obtain biocompounds. In this work, *Spirulina platensis* was used for CO_2 biofixation under different conditions of inorganic carbon. *S. platensis* was inoculated into 200 L bioreactors containing modified Zarrouk's medium (concentration of the carbon was from 1.0 to 2.0 $g.L^{-1}$). CO_2 (12% v/v) was injected into the culture medium intermittently to maintain the inorganic carbon concentration and pH levels favorable for growth. The values of the maximum specific growth rate obtained for both conditions were the same (0.76 day^{-1}). However, the culture in which a concentration of 2.0 $g.L^{-1}$ of $NaHCO_3$ was maintained allowed higher concentrations of biomass (1.0 $g.L^{-1}$) and higher productivity (0.11 $g.L^{-1}.d^{-1}$) to be obtained as compared to the same parameters obtained in cultures containing 1.0 $g.L^{-1}$ of $NaHCO_3$.

Key words: Bioreactor, cyanobacteria, microalgae, *Spirulina*.

INTRODUCTION

The risk of irreversible effects on global climate caused by greenhouse gases has stimulated scientific research on reduced carbon dioxide emissions (Binaghi et al., 2003). Alternatives such as reforestation intensification, fertilizing the ocean with iron and fertilization using major or trace elements (Stewart and Hessami, 2005) are among the technologies studied with the aim of fixing atmospheric carbon in terrestrial or aquatic organisms.

The photoautotrophic growth of microalgae requires supply of CO_2 as source of carbon. At the same time, the supply of CO_2 helps to control the pH of the culture (Radmann et al., 2011). Chemical analyses of the biomass have shown that the microalgal biomass contains 40 to 50% of carbon, suggesting that approximately 1.83 ton of carbon dioxide are required to produce 1.0 ton of biomass (Ho et al., 2011).

Microalgal cultivation technologies have been studied in recent decades in the context of mitigating the emissions of greenhouse gases. The biological fixation of carbon dioxide by microalgae growing in environmental conditions is considered the best way to fix CO_2, because the algae's use of solar energy is much higher than that of terrestrial plants, the maximum photosynthetic capacity of which only lasts for a short period (Rosa et al., 2011).

*Corresponding author. E-mail: jorgealbertovc@terra.com.br. Tel: (+55)(53) 3233-6908.

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

Some of the most noteworthy microorganisms used in the studies of carbon dioxide biofixation are photoautotrophic cyanobacteria, such as *Spirulina platensis*, that fixates CO_2 at a faster rate than eukaryotic organisms. *S. platensis* is a filamentous cyanobacterium capable of forming colonies in tropical or sub-tropical shallow water containing high levels of carbonates and bicarbonates (Lourenço, 2006).

The biomass generated in the CO_2 fixation process has physicochemical characteristics that have a high potential for applicability and can be used in human food or animal feed, for extracting biocomposts (Morais et al., 2015) and obtaining biofuels (Pandey, 2014). *Spirulina* is the most studied microalga and has a proven ability to fix inorganic carbon. This microalga is distinguished for having a generally recognized as safe (GRAS) certificate granted by the Food and Drug Administration (FDA), which ensures its use in food and medicine. Its biomass has a high protein content (64-74%), polyunsaturated fatty acids, pigments and vitamins (Soccol, 2013).

The aim of this study was to use *S. platensis* in a process of carbon dioxide biofixation with different concentrations of inorganic carbon using an open raceway type bioreactor equipped with a carbon dioxide fuel injection system under environmental light conditions.

MATERIALS AND METHODS

Microorganism and culture conditions

This study used the microalga *S. platensis* (*Arthrospira*), as grown in modified Zarrouk culture medium (Zarrouk, 1966). The carbon source ($NaHCO_3$) was added in two concentrations (1.0 and 2.0 $g.L^{-1}$). The cultivations were carried out in 200 L open "raceway" type reactors at 30°C under natural lighting. The inoculum was pre-filtered and rinsed to remove $NaHCO_3$ originating from its propagation (carried out in standard Zarrouk medium, 16.8 $g.L^{-1}$ $NaHCO_3$). CO_2 was injected into the medium at a rate of 10 L CO_2.h in the light phase (when the concentration of $NaHCO_3$ was 1.0 $g.L^{-1}$) and 20 L CO_2.h in the light phase (when the concentration of $NaHCO_3$ was 2.0 $g.L^{-1}$). The light phase was defined as the daily period between 8 and 17 h (9 h per day).

Analytical determinations

The biomass concentration was measured by reading the optical density at 670 nm in a spectrophotometer (Femto 700-Plus, Brazil), with a calibration curve that related optical density to the dry biomass weight; the pH was measured using a digital pH meter (QUIMIS Q400H, Brazil). Ambient and culture medium temperatures were measured with a mercury thermometer.

Assessed kinetic parameters

The maximum concentration of the *S. platensis* biomass (X_{max}, $g.L^{-1}$) was measured, and productivity (P, $g.L^{-1}d^{-1}$) was calculated using Equation 1 (Bailey and Ollis, 1986). The maximum yield (P_{max}) was defined as the highest productivity using Equation 1:

$$P = \left(\frac{X_t - X_0}{t_t - t_0} \right) \quad (1)$$

Where X_t was the cell concentration ($g.L^{-1}$) at time t (d) and X_0 was the cell concentration ($g.L^{-1}$) at time t_0 (d).

The maximum specific growth rate (μ_{max}, d^{-1}) was calculated by the exponential regression of the logarithmic phase of the growth curve for each daily cycle (Bailey and Ollis, 1986).

Calculation of the net efficiency of CO_2 biofixation

The liquid efficiency of biofixation is the ratio between CO_2 fixed in the form of microalgal biomass and the CO_2 transferred to the culture medium, which is given by CO_2 supplied to the system, considering the transfer efficiency of the injection conditions employed. The net efficiency of biofixation is determined by Equation 2:

$$\varphi = \frac{(X_t - X_0) * V * CX * \left(\frac{M_{CO_2}}{M_C} \right)}{\left(Q * Y_{CO_2} * \frac{1}{22,4} * M_{CO_2} * t_I \right) * \varepsilon} \quad (2)$$

where X_t ($g.L^{-1}$) is the biomass concentration at time t (d), X_0 ($g.L^{-1}$) is the biomass concentration at time t_0, CX is the fraction of carbon determined in the microalgal biomass, V (L) is the volume of medium in the photobioreactor, M_{CO2} ($g.mol^{-1}$) and M_C are the molar masses of carbon dioxide and carbon present in the biomasses, respectively, Q is the injected gas flow, Y fraction CO_2 in the gas injected and Ɛ is the transfer efficiency. The percentage of carbon present in biomass (CX) is considered to be 50%, according to Benemann (1997).

Calculating the overall efficiency of the CO_2 biofixation

The overall efficiency of biofixation is the ratio between CO_2 fixed in the form of microalgal biomass and the total CO_2 supplied to the system. The overall efficiency of biofixation was determined using Equation 3.

$$\phi = \frac{(X_t - X_0) * V * CX * \left(\frac{M_{CO_2}}{M_C} \right)}{\left(Q * Y_{CO_2} * \frac{1}{22,4} * M_{CO_2} * t_I \right)} \quad (3)$$

RESULTS AND DISCUSSION

Figure 1 shows the growth of the cyanobacterium *S. platensis* for the two conditions assessed (1 and 2 $g.L^{-1}$ of $NaHCO_3$). In the experiment, the concentration of $NaHCO_3$ of 2.0 $g.L^{-1}$ resulted in the greatest cell concentrations (1.0 $g.L^{-1}$). In cultures carried out with 1.0 $g.L^{-1}$ of $NaHCO_3$, the production rates (via photosynthesis) and consumption of biomasses (via respiration) were equal after the sixth day. This phenomenon is known as the compensation point and occurs when the photosynthetic activity carried out during

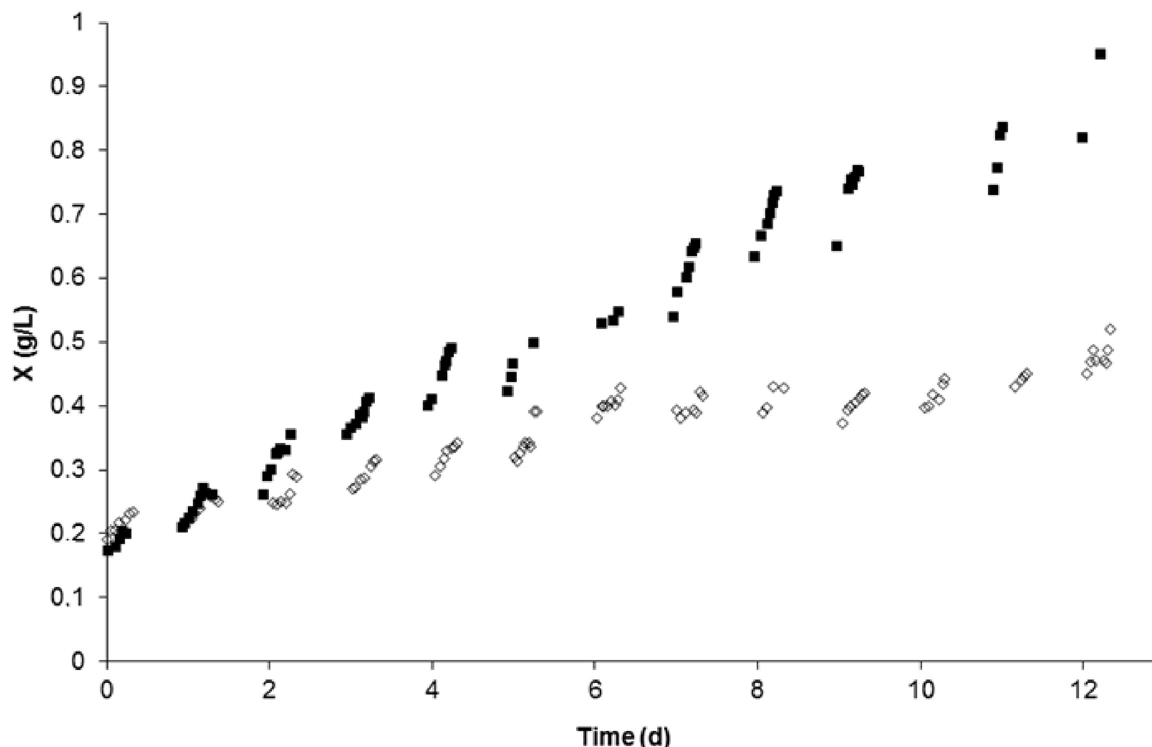

Figure 1. Growth curves of *S. platensis*: $NaHCO_3$ 1.0 g.L^{-1} (◊); $NaHCO_3$ 2.0 g.L^{-1} (■)

Table 1. Kinetic parameters obtained.

$NaHCO_3$ (g.L^{-1})	X_{max} (g.L^{-1})	P_{max} (g.L^{-1}.d^{-1})	μ_{max} (d^{-1})
1.0	0.52	0.07	0.76
2.0	1.00	0.11	0.76

the day equals the heterotrophic activity that occurs during the night. In this assay, the compensation point is associated with the limitation of growth due to lack of nutrients (in this case, the carbon).

When the $NaHCO_3$ concentration was 2.0 g.L^{-1}, the rate grew continuously throughout the cultivation period, and there was no compensation point in this condition. The maximum cell concentration obtained in these experiments was twice as high as that obtained when 1.0 g.L^{-1} of $NaHCO_3$ was added, showing that there is a direct relationship between the concentration of dissolved inorganic carbon with the cellular concentration maintained in the cultures (Table 1).

The bicarbonate concentration in the medium determines the rate of formation of carbon dioxide ($HCO_3^- \xleftrightarrow{k} CO_2 + OH^-$), where k is the kinetic constant of the reaction. The growth rate and cell concentration determine the culture's carbon dioxide demand; thus, the bicarbonate concentration maintained in the medium depends on these parameters. Table 1 presents the kinetic parameters obtained during the experiments. The same maximum specific growth was obtained for both experiments (0.76 day^{-1}), demonstrating that growth is not impaired under any of the conditions. However, the parameter that must be followed in this case is the cell concentration to be maintained, as this metric will determine whether the carbon is excessive (which would cause the loss of carbon into the atmosphere) or in short supply (thus limiting the growth).

Figure 1 show that, during the light phase (day time), growth occur at higher rates than those observed when evaluated over several days of cultivation. This is due to the consumption of the biomass resulting from the activation of the heterotrophic metabolism during the dark phase (night). Under environmental conditions, the cultures are not illuminated during the night. Vonshak and Richmond (1988) reported that the loss of biomass in *Spirulina* cultures caused by respiration during the night can represent 35% of the biomass produced during the day. Figure 2 shows the growth curve determined over the light phase on the fourth day with the medium containing 2.0 g.L^{-1} of $NaHCO_3$. One alternative to significantly increase growth rates would be to keep the cultures illuminated at night, thereby avoiding the consumption of biomass. Another option to avoid the nocturnal loss of carbon is the use of organic carbon sources, such as glucose or acetate, during the night (Ogbonna and Tanaka, 1996). Such measures would ensure biomass growth, even when the heterotrophic

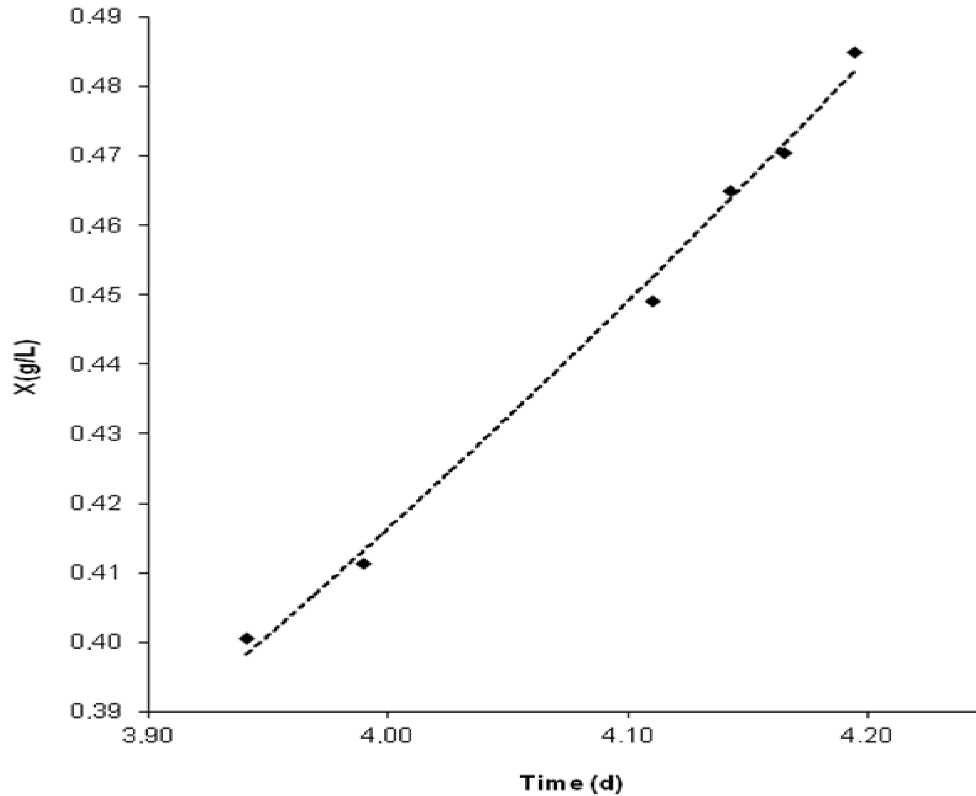

Figure 2. Growth of *S. platensis* during the light phase.

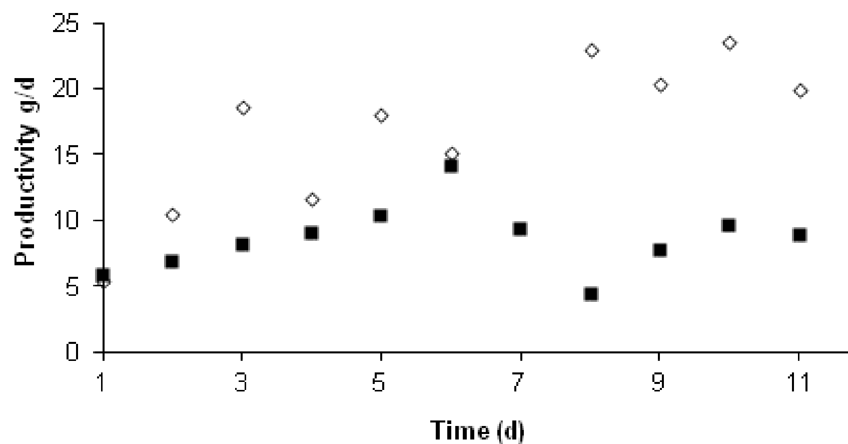

Figure 3. Productivity of cultures containing $NaHCO_3$ 1.0 $g.L^{-1}$ (■) or $NaHCO_3$ 2.0 $g.L^{-1}$ (◊)

(Ogbonna and Tanaka, 1996). Such measures would ensure biomass growth, even when the heterotrophic metabolism is activated, although the consumption of carbon dioxide would be zero during the entire period in which the microalga stopped photosynthesizing (Andrade and Costa, 2007).

The productivity of the culture (Figure 3) increased for the test containing 2.0 $g.L^{-1}$ of $NaHCO_3$ during the study period. However, when 1.0 $g.L^{-1}$ of $NaHCO_3$ was added, the productivity fell after the sixth day of cultivation. On the first day of cultivation, when the cellular concentration was low, the difference in the concentrations of inorganic carbon did not influence the productivity of the cultures. However, when the cell concentration increased, the

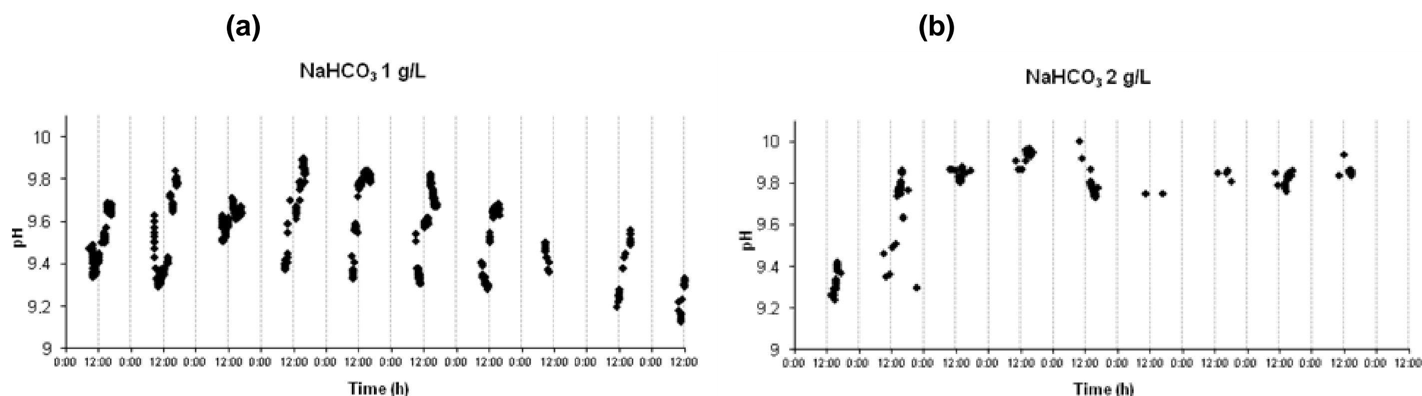

Figure 4. pH of the medium throughout the cultivations.

growth was limited by the carbon source.

In the study by Pelizer et al. (2003), the best results with related to specific growth rate were obtained when the cell concentration used in the inoculum was 50 mg.L^{-1}. However, according to Vonshak et al. (1982), the density of the *Spirulina* population must be 400-500 mg.L^{-1}. These authors showed that the maximum daily production rates of *Spirulina* occurred in this range, with a significant reduction in concentrations above these values.

Radmann et al. (2007) evaluated the blend concentration that must be maintained and the Zarrouk medium dilution in semi-continuous cultivations of *S. platensis*. According to the results obtained in this study, the highest specific growth rates were seen when the blend concentration was 400 mg.L^{-1} in Zarrouk medium containing 20% v/v of the original formulation, which produced a $NaHCO_3$ concentration of 3.36 g.L^{-1}.

Figure 4a shows the behavior of the pH of the medium as a function of time of the cultivation for the experiments with 1.0 g.L^{-1} of $NaHCO_3$. During the light phase, the pH increases, whereas during the dark phase, the pH falls, returning to its initial value. This cycle is due to two phenomena that occur during the daily cycle. During the light phase, the autotrophic metabolism consumes CO_2 and increases the medium's pH; during the dark phase, the heterotrophic metabolism releases CO_2 in the medium, thereby reducing the pH.

Shiraiwa et al. (1993) reported an increase in the pH from 6-9 in the medium of *Chlorella* cultures after just a few minutes in the presence of inorganic carbon (in the form of HCO_3^-) and light. This pH increase has been linked with the carbon consumption and the production of O_2 during photosynthesis. The conversion of HCO_3^- into CO_2 and OH^- was the main cause of the change in the medium's pH. The medium pH is a function of biological activity; thus, during the light phase when carbon dioxide is being consumed, the pH increases. During the night, when the respiration rate causes the release of carbon dioxide, the pH drops.

In the assay containing 2.0 g.L^{-1} of $NaHCO_3$ (Figure 4b), the pH did not follow the same patterns as in the assay with 1.0 g.L^{-1} of $NaHCO_3$; instead, the pH increased during the first days of cultivation although normally it would drop overnight. In this case, the high concentration of inorganic carbon present under these conditions led to a transfer of CO_2 to the external medium, raising the pH. In the experiment during which 2.0 g.L^{-1} of $NaHCO_3$ was initially added, the CO_2 concentration in the medium during the first days of cultivation exceeded the demand for the cultivation. This made the pH to rise, even at night when the photosynthetic activity ceased and when the consumption of biomass would usually decrease the medium's pH.

Richmond and Grobbelaar (1986) studied the relationship between the medium's pH and the purity of the monoalgal culture. They found that, under high pH conditions (above 10.0), *Spirulina* cultures presented a reduction in contamination by other microorganisms. However, during this study, it was found that productivity is maintained at its maximum value between pH 9.5 and 10.5. Above pH 10.5, a sharp decrease in culture productivity can be seen.

The pH controls the growth of *Spirulina*. In fact, at a pH above 10.2 to 10.4, a clear decrease in productivity was noted (Richmond and Grobbelaar, 1986; Vonshak et al. 1982). Experiments conducted on a laboratory scale by Jiménez et al. (2003) showed that the growth rate of *Spirulina* was significantly reduced (15-20%) at a pH above 9.5.

In order to determine the culture conditions that provide the highest efficiency of carbon dioxide fixation, the fixation efficiencies were measured on each day of cultivation. Figure 5 shows the fixation efficiency.

In the experiment containing 1.0 g.L^{-1} of $NaHCO_3$ (Figure 5a), the initial biofixation efficiency was high, because the amount of carbon supplied is relatively low as compared to the amount in the experiment containing 2.0 g.L^{-1} of $NaHCO_3$. After this point, values above 100% can be seen, indicating that the carbon consumption was

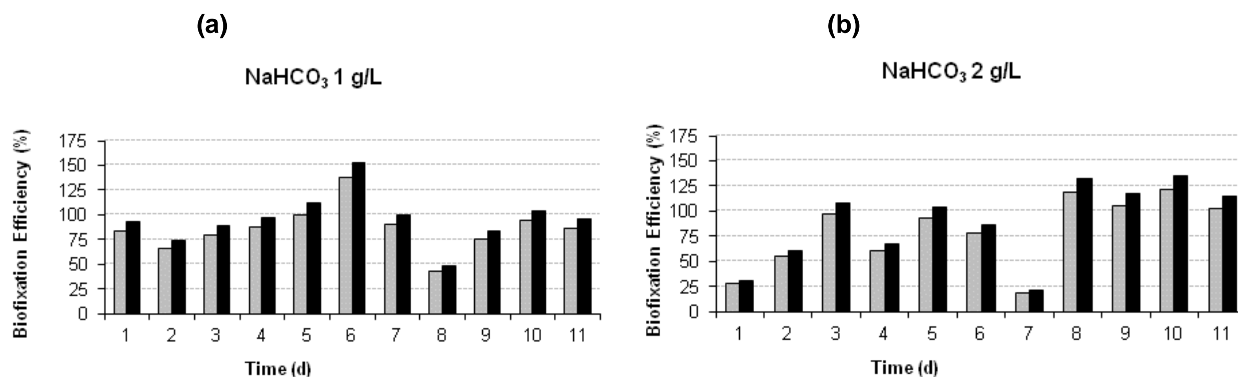

Figure 5. Biofixation efficiency– Overall ; net .

greater than the amount that could be provided by the CO_2 from the injected gas alone. In this case, growth takes place under limited carbon conditions, and the growth rate falls. In the experiment with 2.0 $g.L^{-1}$ of $NaHCO_3$ (Figure 5b), the initial efficiency was low due to the high carbon dioxide concentration, and the best values were achieved after 3 days when the cell concentration reached 0.4 $g.L^{-1}$. After the seventh day of cultivation, the efficiency exceeded 100% (cell concentration of 0.7 $g.L^{-1}$).

Morais and Costa (2007) and Watanabe and Hall (1996) both reported efficiencies of 53.29 and 54% in cultures of S. *platensis* in the tubular photobioreactors. However, in this type of bioreactor, the injection of the air/CO_2 mixture was usually continuous. When the CO_2 supply was carried out on-demand, losses were minimized, and biofixation efficiency increased. In the raceway type of bioreactors, the maximum efficiencies of CO_2 conversion into biomass obtained were approximately 80% (Vonshak and Richmond, 1988). However, these authors showed that high efficiencies are rarely found in large systems, due to the means used to transfer CO_2 to the culture medium.

Conclusions

The maximum concentration of cells in cultures containing 1.0 $g.L^{-1}$ of $NaHCO_3$ was 520 $mg.L^{-1}$. In cultures containing 2.0 $g.L^{-1}$ of $NaHCO_3$, the cell concentrations can be maintained between 800 and 900 $mg.L^{-1}$ with no reduction in productivity, showing the importance of the carbon source for the photosynthetic growth of microalgae. The maximum productivity obtained was 0.13 $g.L^{-1}.d^{-1}$ in the experiment containing 2.0 $g.L^{-1}$ of $NaHCO_3$. Regarding environmental issues, the use of carbon dioxide from gas streams as combustion gases represents an alternative for reducing the emissions of greenhouse gases into the atmosphere. Economically, the use of flue gas as a source of inorganic carbon markedly reduces the costs of the processes required to obtain *Spirulina* biomass.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank the Brazilian Electric Power Company (ELETROBRAS) and the Thermal Generation of Electric Energy Company (CGTEE) for their financial support of this study.

REFERENCES

Andrade MR, Costa JAV (2007). Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate. Aquaculture. 264:130-134.

Bailey JE, Ollis DF (1986). Biochemical. Engineering Fundamentals, McGraw-Hill, New York, P 984.

Benemann JR (1997). CO_2 mitigation with microalgae systems. Energy Convers. Manag. 38:475-479.

Binaghi L, Del Borghi A, Lodi A, Converti A, Del Borghi M (2003). Batch and fed-batch uptake of carbon dioxide by *Spirulina platensis.* Process. Biochem. 38:1341-1346.

Jiménez C, Cossío BR, Niella FX (2003). Relationship between physicochemical variables and productivity in open ponds for the production of *Spirulina*: A predictive model of algal yield. Aquaculture 221:331-345.

Ho SH, Chen CY, Lee DJ, Chang JS (2011). Perspectives on microalgal CO_2-emission mitigation systems - A review. Biotechnol. Adv. 29: 189–98.

Lourenço SO (2006). Cultivo de microalgas marinhas: princípios e aplicações, Editora RiMa, São Carlos, P 606.

Morais MG, Vaz BS, Morais EG, Costa JAV (2015). Biologically Active Metabolites Synthesized by Microalgae. Biomed. Res. Int. 2015:1-15.

Ogbonna JC, Tanaka H (1996). Night biomass loss and changes in biochemical composition of cells during light/dark cyclic culture of *Chlorella pyrenoidosa*. J. Ferment. Bioeng. 82:558-564.

Pandey A, Lee DJ, Chisti Y, Soccol C (2014). Biofuels from algae, Elsevier, San Diego, P 31.

Pelizer LH, Danesi EDG, Rangel CO, Sassano CEN, Carvalho JCM, Sato S, Moraes IO (2003). Influence of inoculum age and concentration in *Spirulina platensis* cultivation. J. Food. Eng. 56:371-375.

Radmann EM, Reinehr CO, Costa JRV (2007). Optimization of the repeated batch cultivation of microalga *Spirulina platensis* in open raceway ponds. Aquaculture 265:118-126.

Radmann EM, Camerini FV, Santos TD, Costa JAV (2011). Isolation and application of SOX and NOX resistant microalgae in biofixation of CO_2 from thermoelectricity plants. Energy Convers. Manag. 52:

3132–3136.
Richmond A, Grobbelaar JU (1986). Factors affecting the output rate of *Spirulina platensis* with reference to mass cultivation. Biomass 10:253-264.
Rosa APC, Carvalho LF, Goldbeck L, Costa JAV (2011). Carbon dioxide fixation by microalgae cultivated in open bioreactors. Energy Convers. Manag. 52: 3071–3073.
Shiraiwa Y, Goyal A, Tolbert NE (1993). Alkalization of the medium by unicellular green algae during uptake of dissolved inorganic carbon. Plant. Cell. Physiol. 34:649-657.
Soccol C, Pandey A, Larroche C (2013). Fermentation Processes Engineering in the Food Industry, CRC Press, USA, P 14.
Stewart C, Hessami MA (2005). A study of methods of carbon dioxide capture and sequestration - The sustainability of a photosynthetic bioreactor approach. Energy Convers. Manag. 46:403-420.
Vonshak A, Abeliovich A, Boussiba S, Arad S, Richmond A (1982). Production of *Spirulina* biomass: Effects of environmental factors and population density. Biomass 2:175-185.
Vonshak A, Richmond A (1988). Mass production of the blue-green alga *Spirulina:* An overview. Biomass 15:233-247.
Watanabe Y, Hall DO (1996). Photosynthetic CO_2 conversion technologies using a photobioreactor incorporating microalgae-energy and material balances. Energy Convers. Manag. 37:1321-1326.
Zarrouk C (1966). Contribution à l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et photosynthese de *Spirulina maxima* geitler. Ph.D. Thesis, Université de Paris.