Biofixation of CO_2 from Synthetic Combustion Gas Using Cultivated Microalgae in Three-Stage Serial Tubular Photobioreactors

Michele G. Morais, Elisângela M. Radmann, and Jorge A. V. Costa*

Laboratory of Biochemistry Engineering, College of Chemistry and Food Engineering, Federal University of Rio Grande (FURG), P. O. Box 474, Rio Grande-RS, 96201-900, Brazil. Fax: +55-53-32338745. E-mail: jorge@pq.cnpq.br

* Author for correspondence and reprint requests

Z. Naturforsch. **66 c**, 313–318 (2011); received September 13, 2010/January 14, 2011

Coal is the most abundant of the fossil fuels, with reserves estimated at 10^2 billions of tons. The feasibility of using coal as a fuel depends upon reducing emissions of gas when it is burnt, such as carbon dioxide (CO_2), sulfur oxides (SO_x), and nitrogen oxides (NO_x). The removal of CO_2 with microalgae may be one of the most efficient ways of reducing this gas, without the need for radical changes in the world's energy supply and production methods. *Spirulina* sp. LEB-18 and *Scenedesmus obliquus* LEB-22 were cultivated in serial tubular photobioreactors, with the aim of measuring the potential of CO_2 biofixation and the resistance of the microalgae to SO_2 and NO. *Spirulina* sp. and *S. obliquus* had CO_2 biofixation scores of 0.27 and 0.22 g L^{-1} d^{-1}, respectively. Both microalgae were resistant to SO_2 and NO, and grew during the 15 d they were cultivated, which proves that using microalgae is an efficient method of biofixation of CO_2 emitted when fossil fuels are burnt.

Key words: Carbon Dioxide, Microalgae, Photobioreactor

Introduction

Although renewable energy sources, such as biomass, solar and wind power, account for the greatest part of the world energy matrix, coal will continue to be the main raw material for energy generation both in developed and developing countries.

Internationally, despite its severe impact on the environment, coal is an important source of energy. The main reasons for this are the vast amount of reserves, its geographic distribution, low costs, and price stability, when compared to other fuels. Coal is responsible for around 7.9% of all world energy consumption and 39.1% of all electrical energy generated (Aneel, 2009).

The burning of coal in industries and thermoelectric power stations has a serious social and environmental impact, due to the emission of particulate and pollutant gases, among which carbon dioxide (CO_2), sulfur dioxide (SO_2), and nitrogen oxide (NO) stand out. In addition to being harmful to human health, these gases are some of the major contributors to the greenhouse effect (CO_2) and acid rain (SO_2 and NO), which provokes the acidification of soil and water, and, consequently, changes in biodiversity. Large-scale use of coal in the future will be linked to advances in the area of research and development, aimed at improving the conversion efficiency, reducing the environmental impact (especially the emission of pollutant gases), and increasing its commercial competitiveness (Aneel, 2009).

Microalgae have been used to capture and assimilate CO_2. While trees fix 1.0–3.5 t CO_2 h^{-1} over the year, microalgae fix 6.3–16.2 t CO_2 h^{-1} (Henrikson, 1994). Using microalgae may be one of the most efficient ways of reducing CO_2 in the atmosphere, without the need for an immediate change in the world energy supply or production methods (Radmann and Costa, 2008).

Vertical tubular photobioreactors increase the amount of time that CO_2 resides in the cultivation medium and, consequently, the CO_2 utilization efficiency (Ono and Cuello, 2004). Cultivation can also be carried out in serial photobioreactors in which unused CO_2 effluent from first photobioreactor is fed into a second photobioreactor. The advantage of this system is that there are lower levels of CO_2 in the final gaseous effluent (Morais and Costa, 2007a).

The objective of this work was to cultivate the microalgae *Spirulina* sp. and *Scenedesmus obliquus* in three-stage serial tubular photobioreactors and to determine the CO_2 fixation potential and resistance to SO_2 and NO.

Material and Methods

Microorganisms and culture media

The *Spirulina* sp. strain LEB-18 (Cyanobacteria, Oscillatoriales) (Morais *et al.*, 2008) and *Scenedesmus obliquus* strain LEB-22 (Chlorophyta, Chlorophyceae) (Morais and Costa, 2007b) were from laboratory stock cultures. This study used carbon-free media for the maintenance and cultivation of both organisms: modified Zarrouk medium for *Spirulina* sp. and MC medium for *S. obliquus* (Morais and Costa, 2007c). For the experiments, inocula of both organisms were acclimatized to carbon dioxide by maintaining them for 7 d under air mixed with 1% (v/v) added carbon dioxide.

Photobioreactors and cultivation conditions

Pure cultures of *Spirulina* sp. and *S. obliquus* were individually cultivated in three 2-L (1.8 L working volume) column photobioreactors (CP) connected in series (Fig. 1). They were labelled, consecutively, as CP1, CP2, and CP3. They were then agitated and aerated using air from a compressor and a sintered sparger. The effluent air (with CO_2, NO and SO_2) from CP1 was fed to the sparger in CP2, and the effluent from this photobioreactor was fed to CP3. The photobioreactors were maintained at 30 °C in a growth chamber under a 12-h dark/light photoperiod. An illumination of 12.8 W m^{-2} was provided by 40-W day-light-type fluorescent lamps (Osram, SP, Brazil) during the light period (Morais and Costa, 2007c).

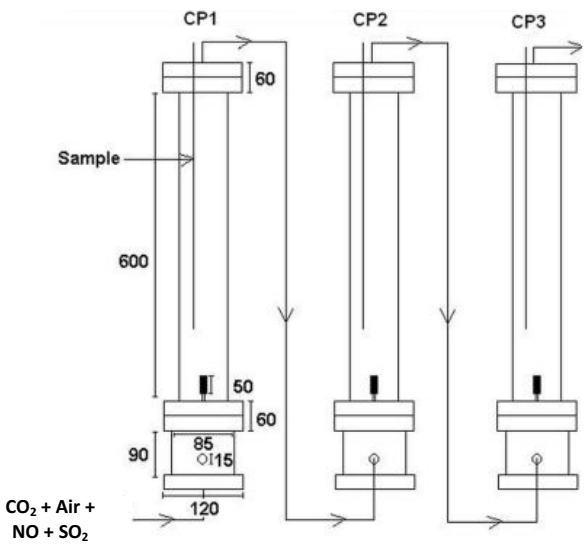

Fig. 1. Three-stage serial photobioreactor scheme. All measurements are in millimeters.

The experiments were carried out by adding either just CO_2 or a mixture of filter-sterilized CO_2, NO, and SO_2 (White Martins, Sapucaia do Sul, Brazil) to the air that entered CP1. The air entered at a rate of 0.3 v v^{-1} min^{-1} (volume gas per volume media per min) for 15 min every 2 h during the 12-h light period, so that the final gas contents entering the media were either 12% CO_2 (v/v), or 12% CO_2 (v/v), 100 ppm NO (v/v), and 60 ppm SO_2 (v/v). The initial biomass concentration in all runs was 0.20 g L^{-1}, and all runs continued for 15 d.

Analytical determinations

Samples of the culture medium were aseptically collected at 24-h intervals. Biomass concentration (X, g L^{-1}) was calculated by measuring the optical density at 670 nm using a 700 Plus spectrophotometer (Femto, SP, Brazil) and a calibration curve of optical density versus dry biomass (Morais and Costa, 2007c). Biomass carbon content was measured using a Perkin Elmer 2400 CHNS (carbon, hydrogen, nitrogen, sulfur) element analyzer calibrated to the 100% value using a certified cystine standard (Perkin Elmer, Waltham, USA).

Kinetic parameters

Biomass concentration (X) values and exponential regression were used to calculate the maximum specific growth rate (μ_{max}, d^{-1}) during the logarithmic phase (Bailey and Ollis, 1986). Doubling time (t_d, d) was calculated as $t_d = \ln 2\ \mu_{max}^{-1}$. Maximum biomass concentration achieved in a photobioreactor was designated X_{max} (g L^{-1}). Productivity (P, g L^{-1} d^{-1}) was obtained using the equation $P = (X_t - X_i)\ (t - t_i)^{-1}$, where X_t is the biomass concentration (g L^{-1}) at time t (d) and X_i is the biomass concentration at the time of inoculation (t_i) (Schmidell *et al.*, 2001). Maximum productivity during cultivation was designated P_{max} (g L^{-1} d^{-1}).

CO_2 biofixation

CO_2 fixation for each microorganism was calculated using the CHNS data. The maximum biofixed CO_2 for the system CP (BF, g L^{-1} d^{-1}) was determined as $BF = (X_t - X_i)\ m_{cbm}\ (M_{CO2}\ M_C^{-1})\ t^{-1}$ where X_t is the biomass concentration (g L^{-1}) at time t (d), X_i is the biomass concentration at the time of inoculation (t_i), m_{cbm} is the mass fraction

Table I. Maximum biomass concentration (X_{max}), maximum productivity (P_{max}), and maximum CO_2 biofixation (BF) for *Spirulina* sp. and *S. obliquus* growing in the three different photobioreactors (CP) which constitute the three-stage serial photobioreactor. Values shown are means ± standard deviation.

CP	X_{max} [g L^{-1}]	P_{max} [g L^{-1} d^{-1}]	BF [g L^{-1} d^{-1}]
Spirulina sp. cultivated with 12% CO_2			
CP1	2.59 ± 0.01	0.18 ± 0.01[a]	0.26 ± 0.01[a]
CP2	2.69 ± 0.02	0.18 ± 0.01[a]	0.26 ± 0.01[a]
CP3	2.76 ± 0.02	0.17 ± 0.01[a]	0.29 ± 0.01[b]
S. obliquus cultivated with 12% CO_2			
CP1	1.77 ± 0.01	0.18 ± 0.01[a]	0.28 ± 0.01[b]
CP2	1.42 ± 0.02[a]	0.14 ± 0.01	0.21 ± 0.01
CP3	1.32 ± 0.01[bc]	0.10 ± 0.01[b]	0.16 ± 0.01
Spirulina sp. cultivated with 12% CO_2, 60 ppm SO_2, 100 ppm NO			
CP1	1.42 ± 0.01[a]	0.10 ± 0.01[b]	0.14 ± 0.01[c]
CP2	1.35 ± 0.01[b]	0.09 ± 0.01[c]	0.13 ± 0.01[c]
CP3	1.29 ± 0.01[c]	0.09 ± 0.01[c]	0.13 ± 0.01[c]
S. obliquus cultivated with 12% CO_2, 60 ppm SO_2, 100 ppm NO			
CP1	0.64 ± 0.01	0.15 ± 0.01	0.14 ± 0.01[c]
CP2	0.99 ± 0.01	0.09 ± 0.01[c]	0.09 ± 0.01
CP3	0.77 ± 0.01	0.04 ± 0.01	0.08 ± 0.01

Values with the same letters in the same column indicate that the values did not differ when using Tuckey's test ($p \leq 0.10$).

of carbon in grams of carbon per gram of biomass (g g^{-1}), M_{CO2} (g mol^{-1}) is the molar mass of CO_2 and M_C (g mol^{-1}) is the molar mass of carbon (Morais and Costa, 2007c).

Statistical analysis

The experimental results were evaluated by analysis of variance (ANOVA) of the kinetic parameters and biofixation of the system medium of three-stage serial tubular photobioreactors. Significance levels were assessed using Tuckey's test at $p \leq 0.10$.

Results and Discussion

Growth parameters and characteristics of CO_2 fixation in each photobioreactor of the series

Table I shows the growth kinetics and CO_2 fixation for *Spirulina* sp. and *S. obliquus* in each of the three photobioreactors in the system. The highest cellular concentration [(2.76 ± 0.02) g L^{-1}] was reached when 12% CO_2 was added to *Spirulina* sp. ($p < 0.001$), especially in photobioreactor CP3 (Fig. 1). *Spirulina* sp. and *S. obliquus* reached the highest cellular concentrations ($p = 0.0001$) under conditions where only CO_2 was added, when compared with the trials carried out with synthetic gases. Maximum concentrations are important to obtain high cellular density, which makes the cultivation of microalgae with high operational costs economically feasible.

The productivity was highest ($p < 0.001$) in CP1 in the *S. obliquus* group with the addition of CO_2 alone or with CO_2, SO_2, and NO. Apart from the *Spirulina* sp. group with the addition of just CO_2, the highest productivity in all the other conditions ($p < 0.001$) was always observed in CP1 of the series.

Air, CO_2 and, when present, SO_2 and NO were fed into the series of photobioreactors, starting with CP1, so the first photobioreactor in the series received the highest quantity of gases, compared to the subsequent one which was fed with the effluent from the previous one. Because the microalgae had the highest P_{max} in CP1, it can be confirmed that addition of SO_2 and NO does not affect the growth of the studied microalgae, so these can be grown in electrical energy plants to biofix CO_2 released in coal combustion, and thereby reduce global warming.

Spirulina sp. cultivated with just 12% CO_2 added to the air had the highest biofixation rate in CP3 ($p < 0.001$). The amount of carbon dioxide in air is normally 0.038% and is optimal for the growth of higher plants (not true for C_3 plants), although some higher plants can tolerate up to 0.1%. Many phototrophic microorganisms can grow at, or above, 12% atmospheric CO_2 (Pulz,

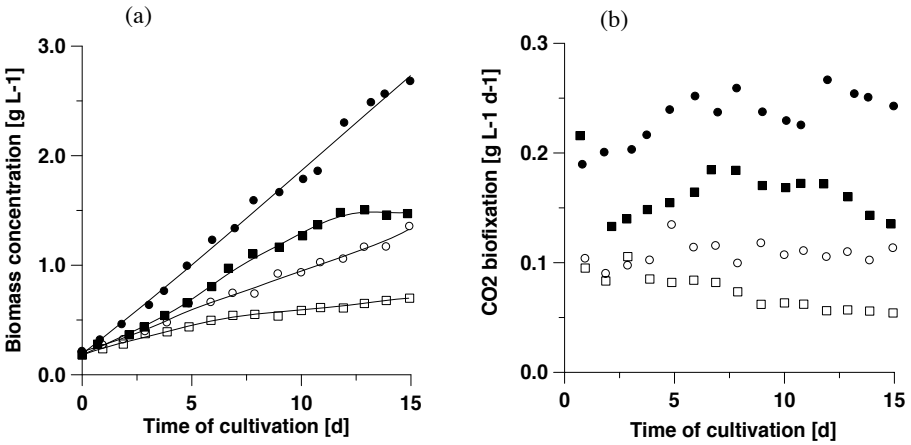

Fig. 2. Time course of (a) biomass concentration and (b) CO_2 biofixation in three-stage serial photobioreactors in the presence of 12% CO_2 (v/v) with *Spirulina* sp. (●) and *S. obliquus* (■), and 12% CO_2 (v/v), 60 ppm SO_2 (v/v), and 100 ppm NO (v/v) with *Spirulina* sp. (○) and *S. obliquus* (□).

2001). This is supported by the present study, which showed that *Spirulina* sp. and *S. obliquus* can grow under atmospheres containing 12% CO_2. Lee *et al.* (2002) pointed out that synthetic flue gases contain 12% to 15% CO_2, so it seems possible that *Spirulina* sp. and *S. obliquus*, which, as the results of the present study suggest, can grow at 12% CO_2, could be used to mitigate the production of carbon dioxide by fossil-fuel combustion.

When cultivated with CO_2, NO, and SO_2, *Spirulina* sp. had the same rate of CO_2 fixation in all three of the photobioreactor series. *S. obliquus*, with or without the addition of NO and SO_2, had the maximum rate of CO_2 fixation ($p < 0.001$) in the first photobioreactor of the series.

Growth parameters and characteristics of CO_2 fixation in the three-stage serial photobioreactors

The growth curves for *Spirulina* sp. and *S. obliquus* (Fig. 2a) show no adaptation (lag) phase because the stock cultures had been maintained below 1% (v/v) CO_2. According to Schmidell *et al.* (2001), the initial sample (inoculum) adaptation determines the presence or the absence of the lag phase. Yun *et al.* (1997) confirmed that the inoculum of *Chlorella vulgaris* which had adapted to 5% CO_2 grew better in a culture with 15% of gas as compared to the inoculum without acclimatization.

From Fig. 2a, it can be seen that in cultures, to which just CO_2 was added, the microalgae grew most, when compared with the trials with synthetic gas. *Spirulina* sp. had greater rates of cellular concentration than *S. obliquus*, in both the culture with added CO_2 and in the trial with CO_2, SO_2, and NO. Comparing Fig. 2a and 2b, it can be seen that the trials with highest growth also had higher rates of CO_2 biofixation, and the synthetic gas cultures had the lowest values.

Table II shows the kinetic responses, the length of the exponential growth phase, and the correlation coefficient of the exponential growth phase with the system of three serial photobioreactors. The cultures to which only CO_2 was added had better kinetic and biofixation results than those utilizing CO_2, NO, and SO_2. X_{max} of the photobioreactor system was 2.69 g L^{-1} when *Spirulina* sp. was cultivated with CO_2 ($p < 0.01$). X_{max} of *S. obliquus* cultivated with just CO_2 did not differ from that of *Spirulina* sp. cultivated with CO_2, NO, and SO_2 ($p = 0.54$). In these conditions, *S. obliquus* and *Spirulina* sp. had an X_{max} value of 1.35 and 1.51 g L^{-1}, respectively. The maximum cellular concentration of *S. obliquus* cultivated just with added CO_2 was 1.51 g L^{-1}. In culture with added CO_2 in an open circular tank, Binaghi *et al.* (2003) obtained a maximum cellular concentration of 3.4 g L^{-1} for *Spirulina platensis*.

When cultivated under the same conditions, *Spirulina* sp. and *S. obliquus* had no significant difference in their maximum productivity values ($p = 0.23$). The *Spirulina* sp. productivity varied between 0.09 and 0.18 g L^{-1} d^{-1} and for *S. obliquus* between 0.07 and 0.14 g L^{-1} d^{-1}, both with the addition of CO_2 only. Cultures with added CO_2, NO,

Table II. Maximum CO_2 biofixation (*BF*), maximum productivity (P_{max}), maximum specific growth rate (μ_{max}), doubling time (t_d), length of exponential growth phase (Δt), and correlation coefficient of the exponential growth phase (r^2) with the system of three-stage serial tubular photobioreactors. Values shown are means ± standard deviation.

Run	*BF* [g L^{-1} d^{-1}]	P_{max} [g L^{-1} d^{-1}]	μ_{max} [d^{-1}]	t_d [d]	Δt [d]	r^2
SP1	0.27 ± 0.02[a]	0.18 ± 0.01[a]	0.11 ± 0.01[a]	6.3 ± 0.6[a]	3–15	0.961
SC1	0.22 ± 0.06[ab]	0.14 ± 0.04[a]	0.15 ± 0.01	4.6 ± 0.3	1–12	0.957
SP2	0.13 ± 0.01[b]	0.09 ± 0.01[b]	0.09 ± 0.01[a]	7.7 ± 0.9[a]	2–15	0.962
SC2	0.11 ± 0.03[b]	0.07 ± 0.02[b]	0.04 ± 0.01	17.3 ± 4.7	5–15	0.977

SP1, *Spirulina* sp. cultivated with 12% CO_2 (v/v); SC1, *S. obliquus* cultivated with 12% CO_2 (v/v); SP2, *Spirulina* sp. cultivated with 12% CO_2 (v/v), 60 ppm SO_2 (v/v), 100 ppm NO (v/v); SC2, *S. obliquus* cultivated with 12% CO_2 (v/v), 60 ppm SO_2 (v/v), 100 ppm NO (v/v). Values with the same letters in the same column indicate that the values did not differ when Tuckey's test was used ($p \leq 0.10$).

and SO_2 had a maximum productivity of 0.09 and 0.07 g L^{-1} d^{-1} for *Spirulina* sp. and *S. obliquus*, respectively. Maximum productivity takes into account the relationship between cellular concentration and cultivation time, which indicates the performance of a process and can define the length of time a discontinuous cultivation takes to produce biomass.

Maximum values of the specific growth rate of *Spirulina* sp. *and S. obliquus*, in trials with CO_2 added only, were (0.11 ± 0.01) d^{-1} and (0.15 ± 0.01) d^{-1}, respectively. In this study, the cultures that were grown for 15 d and with 12% CO_2 added had an exponential growth phase which lasted 12 and 11 d for *Spirulina* sp. and *S. obliquus*, respectively.

The maximum specific growth rate increases when the doubling time decreases and cultivation becomes more economical. Microalgae can duplicate their biomass within days, whereas higher plants may take many months or years (Vonshak *et al.*, 1982).

The results show that adding SO_2 and NO influenced the growth and CO_2 biofixation rates of *Spirulina* sp. However, the concentration of this microalga did not decline during the 15 d of cultivation. This proves that it has the potential to biofix CO_2 derived from the burning of fossil fuels. The lowest generation times were observed in trials where only CO_2 was added.

Sodium bicarbonate is the nutrient added in the highest quantity in the Zarrouk culture medium (16.8 g L^{-1}), which was used for the *Spirulina* sp. microalga; it corresponds to 60% of the total costs of nutrients (Alava *et al.*, 1997). Microalgae that fix the CO_2 can be used to reduce the costs of nutrients and the diverse environmental problems caused by the increase in concentration of this gas in the atmosphere.

Concerning the maximum rate of CO_2 biofixation, the microalgae cultivated with just CO_2 did not significantly differ ($p = 0.32$), reaching 0.27 and 0.22 g L^{-1} d^{-1} for *Spirulina* sp. and *S. obliquus*, respectively. *S. obliquus* was not different ($p > 0.02$) in terms of growth and CO_2 biofixation, when SO_2 and NO were added to the culture. The microalga *S. obliquus* LEB-22 probably had a greater tolerance to the synthetic combustion gas as it had been selected and isolated from a lake located near a coal power station (Morais and Costa, 2007b).

Conclusions

The microalgae cultivated with synthetic combustion gas in tubular photobioreactors connected in series showed potential for CO_2 biofixation, reaching fixation rates of 0.27 and 0.22 g L^{-1} d^{-1} for *Spirulina* sp. and *S. obliquus*, respectively. *Spirulina* sp. and *S. obliquus* had specific maximum growth rates of (0.11 ± 0.01) d^{-1} and (0.15 ± 0.01) d^{-1}, respectively. The maximum productivities were (0.18 ± 0.01) g L^{-1} d^{-1} and (0.14 ± 0.04) g L^{-1} d^{-1} for *Spirulina* sp. and *S. obliquus*, respectively. During the 15 d of cultivation there was no decline in cell density, which shows that the microalgae were SO_2- and NO-resistant. This proves the efficiency of microalgae use for biofixation of CO_2 produced during the burning of fossil fuels.

Acknowledgements

The authors wish to thank the Centrais Elétricas Brasileiras S. A. (ELETROBRÁS) and the Companhia de Geração Térmica de Energia Elétrica (CGTEE) for their financial support of this work.

Alava D., Mello P. C., and Wagner K. (1997), The relevance of the CO_2 partial pressure of sodium bicarbonate solutions for the mass cultivation of the microalga *Spirulina*. J. Braz. Chem. Soc. **8**, 447–450.

Aneel (Agência Nacional de Energia Elétrica) (2009), http://Aneel.gov.br/aplicacoes/atlas/pdf/08-carvao(2).pdf.

Bailey J. E. and Ollis D. F. (1986), Biochemical Engineering Fundamentals, 2nd ed. McGraw-Hill, Singapore.

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

Henrikson R. (1994), Microalga *Spirulina*: Superalimento del futuro. Ediciones S. A. Urano, Barcelona.

Lee J. S., Kim D. K., Lee J. P., Park S. C., Koh J. H., Cho H. S., and Kim S. W. (2002), Effects of SO_2 and NO on growth of *Chlorella* sp. KR-1. Bioresour. Technol. **82**, 1–4.

Morais M. G. and Costa J. A. V. (2007a), Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. J. Biotechnol. **129**, 439–445.

Morais M. G. and Costa J. A. V. (2007b), Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. Energy Convers. Manage. **48**, 2169–2173.

Morais M. G. and Costa J. A. V. (2007c), Carbon dioxide biofixation with *Chlorella kessleri*, *C. vulgaris*, *Scenedesmus obliquus* and *Spirulina* sp. cultivated in flasks and vertical tubular photobioreactors. Biotechnol. Lett. **29**, 1349–1352.

Morais M. G., Reichert C. C., Dalcanton F., Durante A. J., Marins L. F. F., and Costa J. A. V. (2008), Isolation and characterization of a new *Arthrospira* strain. Z. Naturforsch. **63c**, 144–150.

Ono E. and Cuello J. L. (2004), Design parameters of solar concentrating systems for CO_2 mitigating algal photobioreactors. Energy **29**, 1651–1657.

Pulz O. (2001), Photobioreactors: production systems for phototrophic microorganisms. Appl. Microbiol. Biotechnol. **57**, 287–293.

Radmann E. M. and Costa J. A. V. (2008), Conteúdo lipídico e composição de ácidos graxos de microalgas expostas aos gases CO_2, SO_2 e NO. Quím. Nova **31**, 1609–1612.

Schmidell W., Lima A. U., Aquarone E., and Borzani W. (2001), Industrial Biotechnology, Vol. 2. Edgard Blücher LTDA, São Paulo.

Vonshak A., Abeliovich A., Boussiba A., Arad S., and Richmond A. (1982), Production of *Spirulina* biomass: effects of environmental factors and population density. Biomass **10**, 175–185.

Yun Y. S., Lee S. B., Park J. M., Lee C. I., and Yang J. W. (1997), Carbon dioxide fixation by algal cultivation using wastewater nutrients. J. Chem. Technol. Biotechnol. **69**, 451–455.