

CO₂ Biofixation by the Cyanobacterium *Spirulina* sp. LEB 18 and the Green Alga *Chlorella fusca* LEB 111 Grown Using Gas Effluents and Solid Residues of Thermoelectric Origin

Bruna da Silva Vaz¹ · Jorge Alberto Vieira Costa² · Michele Greque de Morais¹

Received: 7 August 2015 / Accepted: 25 September 2015 /
Published online: 9 October 2015
© Springer Science+Business Media New York 2015

Abstract The concentration of carbon dioxide (CO₂) in the atmosphere has increased from 280 to 400 ppm in the last 10 years, and the coal-fired power plants are responsible for approximately 22 % of these emissions. The burning of fossil fuel also produces a great amount of solid waste that causes serious industrial and environmental problems. The biological processes become interesting alternative in combating pollution and developing new products. The objective of this study was to evaluate the CO₂ biofixation potential of microalgae that were grown using gaseous effluents and solid residues of thermoelectric origin. The microalgae *Chlorella fusca* LEB 111 presented higher rate of CO₂ biofixation (42.8 %) ($p < 0.01$) than did *Spirulina* sp. LEB 18. The values for the CO₂ biofixation rates and the kinetic parameters of *Spirulina* and *Chlorella* cells grown using combustion gas did not differ significantly from those of cells grown using CO₂ and a carbon source in the culture media. These microalgae could be grown using ash derived from coal combustion, using the minerals present in this residue as the source of the essential metals required for their growth and the CO₂ derived from the combustion gas as their carbon source.

Keywords Ash · Carbon dioxide · *Chlorella fusca* LEB 111 · Industrial residues · Microalgae · *Spirulina* sp. LEB 18

✉ Michele Greque de Morais
michele.morais@pq.cnpq.br

¹ Laboratory of Microbiology and Biochemical, College of Chemistry and Food Engineering, Federal University of Rio Grande, P.O. Box 474, Av. Itália Km 8, 96203-900 Rio Grande, RS, Brazil

² Laboratory of Biochemical Engineering, College of Chemistry and Food Engineering, Federal University of Rio Grande, P.O. Box 474, Av. Itália Km 8, Rio Grande 96203-900 RS, Brazil

Introduction

The concentration of atmospheric CO₂ has increased 6.5 % annually since 1800, with the emissions of thermoelectric power plants accounting for approximately 22 % of this increase [1]. The President Medici Thermoelectric Power Plant (UTPM), which is located in Candiota (RS, Brazil), has a maximal capacity of 796 MW. Currently, the power plant operates at 50 % maximum capacity, not to exceed the emission limits allowed by Brazilian legislation. An average of 2.234 million tons of CO₂ is emitted annually from the UTPM due to the burning of coal [2].

The coal combustion process also produces solid residues, which have limited utilization and/or disposal options [3]. The amount of ash produced through coal combustion causes difficulties in the heat-transfer process due to the low calorific value of the coal-rich ash available during the phase-separation (solid-gas) process as well as difficulties in the transport and disposal of this residue, which generates major industrial and environmental problems [4].

Many techniques have been applied to reduce the emission of CO₂ into the atmosphere, including physical and chemical treatments using renewable energy sources or carbon-sequestration methods. An alternative to reducing CO₂ emission without changing the global energy matrix is biofixation by microalgae. The photoautotrophic activity of these microorganisms converts CO₂ into biomass. In addition to minimizing the environmental problems caused by CO₂ emission, using CO₂ as a nutrient source reduced the cost of its processing [5–7].

The major components of coal ash are silica (SiO₂), alumina (Al₂O₃), iron oxide (Fe₂O₃), calcium oxide (CaO), and trace mineral elements (As, Co, Ba, Zn, Ni, Cr, Fe, S, Mo, Mn, Cu, etc.) [8]. Many minerals present in coal ash [9] can partially replace the nutrients needed for microalgal growth.

Growing microalgae using the byproducts of coal combustion is a sustainable alternative option that has attracted the attention of many researchers due to the ecological, economical, and nutritional importance of these species and their ability to reduce the emission of pollutants into the environment, with the consequent reduction in the costs of nutrients necessary for their growth and their manufacture of important bioproducts, such as carotene, phycocyanin, biofuels, and biopolymers [7, 10–12].

The objective of this study was to evaluate CO₂ biofixation by *Spirulina* sp. LEB 18 and *Chlorella fusca* LEB 111 microalgal cells grown using gaseous effluents and solid residues of thermoelectric origin.

Materials and Methods

Microorganisms and Culture Medium

Spirulina sp. LEB 18 microalgal cells, which were isolated from the Mangueira lagoon (latitude 33°31'08"S and longitude 53°22'05"W) [13], and *Chlorella fusca* LEB 111 cells, which were isolated from the wastewater and residue-stabilization pond of the UTPM (latitude 24°36'13"S and longitude 52°32'43"W) (Candiota, RS), were used in this study.

The *Spirulina* sp. LEB 18 cells were grown using Zarrouk medium [14] with the following composition (g L⁻¹): NaHCO₃ (16.8); K₂HPO₄ (0.50); NaNO₃ (2.50); K₂SO₄ (1.00); NaCl (1.00); MgSO₄·7H₂O (0.20); CaCl₂ (0.04); FeSO₄·7H₂O (0.01); EDTA (0.08); H₃BO₃ (2.86);

MnCl₂·4H₂O (1.81); ZnSO₄·7H₂O (0.222); CuSO₄·5H₂O (0.079); Na₂MoO₄ (0.015); em (mg L⁻¹), NH₄VO₃ (22.86); K₂Cr₂(SO₄)₄·24H₂O (96); NiSO₄·7H₂O (47.85); Na₂WO₄·2H₂O (17.94); TiOSO₄·H₂SO₄·8H₂O (61.1); Co(NO₃)₂·6H₂O (43.98). The *Chlorella fusca* LEB 111 cells were grown using BG11 medium [15] with the following composition (g L⁻¹): NaNO₃ (1.50); K₂HPO₄·3H₂O (0.04); MgSO₄·7H₂O (0.075); CaCl₂·2H₂O (0.036); ammonium ferric citrate (0.006); EDTA (0.001); Na₂CO₃ (0.02); citric acid (0.006); ZnSO₄·7H₂O (0.222); MnCl₂·4H₂O (1.81); Na₂MoO₄·2H₂O (0.390); H₃BO₃ (2.86); CuSO₄·5H₂O (0.079); and Co(NO₃)₂·6H₂O (0.0494).

Modified culture media were used, in which the original carbon source of the Zarrouk medium (16.8 g L⁻¹ sodium bicarbonate—NaHCO₃) or the BG11 medium (0.02 g L⁻¹ of sodium carbonate—Na₂CO₃) was fully replaced by commercial gas containing 10 % (v/v) CO₂ [16] or by coal-combustion gas. To adapt the microalgae to CO₂, the inocula were maintained for 7 days in the afore-mentioned media in which the carbon source was replaced by 1 % (v/v) CO₂.

Combustion Gas and Ashes

The combustion gas and solid residues (mineral coal ash) used were provided by the UTPM. The coal combustion gas was collected from a tap near the gas emission chimney, coincident with the phase with a power level of 126 MW. This gas was compressed into a cylinder using a portable compressor operated at a pressure level of 10 bar. The ashes were collected in silos, in which they were captured using electrostatic filters to meet the residue-emission standards established by the environmental licensing bodies [2]. The average concentration of the flue gas normally emitted by CGTEE and chemical composition of the ash are shown in Tables 1 and 2, respectively (data provided by the Department of Environment—CGTEE).

Growth Conditions

Microalgal culturing was conducted discontinuously in closed 2 L photobioreactors with a working volume of 1.8 L (using sterile distilled water to replace the evaporated liquid), using an initial microalgal concentration of 0.2 g L⁻¹. The temperature was maintained at 30 °C in a thermostatic chamber, with a 12 h light/dark photoperiod [17] and 43.2 μmol m⁻² s⁻¹ of illuminance provided by fluorescent lamps (General Electric). The cultures were stirred using sterile air mixed with the gas from the industrial cylinder (White Martins, Brazil) containing 10 % (v/v) CO₂ or combustion gas, at a flow rate of 0.05 vvm. The air was injected into the cultures through a porous sprayer curtain (1.0 cm in diameter and 10.0 cm long) that was fixed to the base of the bioreactor. The gases were added to the cultures for 10 min every 2 h during the light growth period. The ashes were added to the culture media to a concentration of 40 ppm. The experiments were performed in duplicate and lasted 12 days.

Table 1 Composition of flue gas

Components	Composition
CO ₂	9.40 % (v/v)
NO _x	400 mg Nm ⁻³
SO _x	5000 mg Nm ⁻³
Ashes	650 mg Nm ⁻³

Table 2 Chemical composition of the utilized coal fly ash

Components	Composition (%)
SiO ₂	65.8
Al ₂ O ₃	21.5
Fe ₂ O ₃	4.6
TiO ₂	0.7
P ₂ O ₅	<0.03
CaO	1.8
MgO	0.8
Na ₂ O	0.1
K ₂ O	1.9
SO ₃	1.4
Loss on ignition	1.4

Analytical Determinations

The biomass concentration was determined daily by relating the optical density of the cultures at 670 nm [7], obtained using a spectrophotometer (QUIMIS Q798DRM), to the values in a calibration curve that correlated the optical densities and the dry weights of the biomasses of each microalga used [18]. The blank values obtained (culture media containing ash) showed that the ash did not interfere in the optical density and/or colorimetry (0 absorbance/100 % transmittance). The pH of the cultures was determined every 24 h using a digital pH meter (LUTRON PH-221). The concentrations of carbon, hydrogen, and nitrogen in the biomasses obtained in each experiment were determined using an elemental CHNS/O analyzer (Perkin-Elmer 2400, USA). The default certified acetanilide specimen was used to calibrate the instrument (Perkin Elmer, USA). The heavy metal analysis was performed using inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin Elmer 2100 DV) of the samples following their acidic digestion in a microwave oven [19]. The quantification of metals was determined in experiments with the maximum biomass concentration and in experiments using standard culture medium for each microalga.

Kinetic Parameters

The biomass concentration values were used to determine the maximal specific growth rates (μ_{\max} , day⁻¹), maximal cell concentrations (X_{\max} , g L⁻¹), and maximal yields (P_{\max} , g L⁻¹ day⁻¹). The highest yield was calculated using Eq. 1, where X_t was the biomass concentration (g L⁻¹) at time t (days) and X_0 was the biomass concentration (g L⁻¹) at time t_0 (days) [20]. The maximal specific growth rate (μ_{\max} , day⁻¹) was obtained by applying exponential regression to the values for the logarithmic phase, according to Eq. 2 [21].

$$P = \frac{X_t - X_0}{t - t_0} \quad (1)$$

$$\mu = \frac{1}{x} \frac{dX}{dt} \quad (2)$$

Carbon Dioxide Fixation

The accumulation of fixed CO₂ (FA, g_{CO2}) was calculated according to Eq. 3, where X_t (g L⁻¹) is the biomass concentration at time t (days), X_0 (g L⁻¹) is the biomass concentration at time t_0 (days), x_{cbm} (g/g_{sample}) is the fraction of carbon determined in the microalgal biomass, V_{FBRT} (L) is the volume of medium in the photobioreactor, and M_{CO_2} (g mol⁻¹) and M_C are the molar masses of carbon dioxide and carbon present in the biomasses, respectively. The daily CO₂ fixation rate (FD, g_{CO2 fixed}/g_{CO2 injected} day) was determined according to Eq. 4, where $FA_{(t+1)}$ is the CO₂ accumulated at time $t+1$ (day), FA_t is the CO₂ accumulated at time t (day), and m_{id} (g_{CO2}) is the mass of CO₂ injected daily. The maximal daily CO₂ fixation rate (F_{CO_2}) is the maximal value achieved during the growth period.

$$FA = (X_t - X_0) * x_{cbm} * V_{FBRT} * \left(\frac{M_{CO_2}}{M_C} \right) \quad (3)$$

$$FD = \frac{(FA_{(t+1)} - FA_t)}{m_{id}} \quad (4)$$

Statistical Analysis

The experimental results were evaluated using an analysis of variance (ANOVA) and Tukey's test to compare the mean values of the kinetic parameters, with the significance level set to 99 % ($p \leq 0.01$).

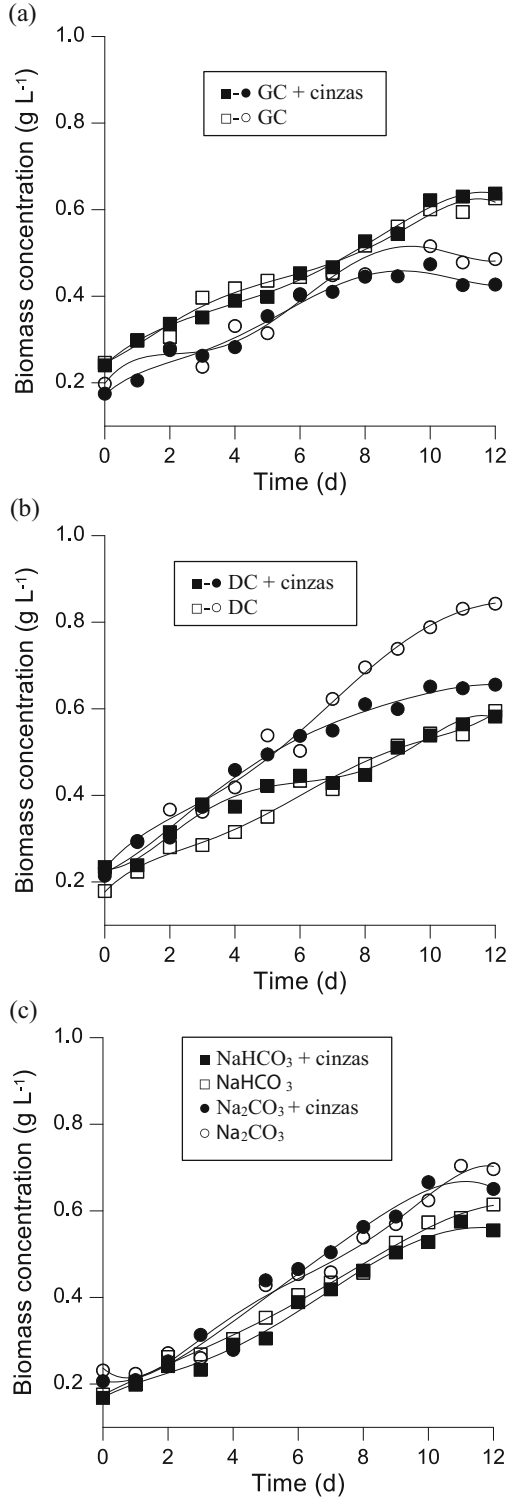
Results and Discussion

Following inoculation, the microalgae grew at an approximately constant rate. The curves of the biomass concentration as a function of the culturing period (Fig. 1) showed no "lag" growth phase [20]. This acclimatization phase was not observed due to the pre-adaptation of the inocula. The growth curve of *Chlorella fusca* LEB 111 microalgal cells (Fig. 1b) cultured using CO₂ revealed a sharp exponential rate increase at the sixth day of culture, after which the growth rate was constant and maximal. The maximal biomass concentration of this strain was obtained under these conditions.

In the experiments conducted using *Spirulina* sp. LEB 18 cells, the maximal biomass concentration was obtained using combustion gas and coal residues. In the experiments conducted using only combustion gas, a biomass concentration of 0.63 g L⁻¹ was obtained, with no significant difference ($p > 1.00$) from that obtained using combustion gas and residues. The ashes contained minerals present in the Zarrouk culture medium that are known to stimulate the microalgal growth.

The *Spirulina* sp. LEB 18 cells reached a higher concentration of biomass when grown using combustion gas with or without ash compared with that of *Chlorella fusca* LEB 111 cells due to the greater availability of sodium bicarbonate obtained from CO₂, which was favored by the pH (10.5) value of the culture medium. Bicarbonate ions enter cells via active transport, after which the carbonic-anhydrase enzyme acts on them, releasing CO₂. The CO₂ molecules

Fig. 1 Growth curves of *Spirulina* sp. LEB 18 (■-□) and *Chlorella fusca* LEB 111 (●-○) microalgal cells grown using combustion gas (CG), carbon dioxide (CO₂), sodium bicarbonate (NaHCO₃), sodium carbonate (Na₂CO₃), and 40 ppm ash



then dissociate to form bicarbonate (HCO_3^-) and H^+ ions, and these bicarbonate ions can dissociate to form CO_2 and carbonate ions (CO_3^{2-}), depending on the pH of the culture medium [22, 23].

The *Spirulina* sp. LEB 18 cells grown using only the combustion gas or the combustion gas and the ashes achieved a higher X_{max} than did the *Chlorella fusca* LEB 111 cells grown under the same conditions. Under other conditions, the values for the kinetic parameters of *Chlorella fusca* LEB 111 microalgal cells were higher than were those of *Spirulina* sp. LEB 18 cells. The values for the kinetic parameters observed using *Chlorella fusca* LEB 111 cells grown using combustion gas alone or with ashes were not significantly different from those of cultures grown using CO_2 or the standard medium.

In addition to CO_2 , the combustion gas contained molecules that are known to inhibit microalgal growth, such as sulfur (SO_x) and nitrogen (NO_x) oxides [24, 25]; however, in this study, it was observed that the *Chlorella fusca* LEB 111 microalgal cells were not affected by these factors. The *Chlorella fusca* cells showed higher rates of CO_2 biofixation than did the *Spirulina* sp. LEB 18 cells, reaching a maximum of 42.8 % when grown using CO_2 . The CO_2 biofixation rates of cultures grown using combustion gas, with or without ash, were not significantly different ($p < 0.01$) from those obtained using CO_2 (Table 3).

The adaptation of *Chlorella fusca* LB 111 to the combustion gas and ash can be explained by this strain having been isolated from a region near the UTPM and thus having been exposed to its emissions. Native species of microalgae have been reported to be more tolerant of local conditions and to exhibit higher rates of photosynthesis and biomass production [26–29]. Microalgae isolated from this region exhibit maximally efficient CO_2 biofixation. The environmental impact of the high concentrations of combustion gases and solid residues produced

Table 3 Maximal biomass concentration (X_{max} , g L^{-1}), maximal yield (P_{max} , $\text{g L}^{-1} \text{ day}^{-1}$), maximal specific growth rate (μ_{max} , day^{-1}), maximal daily CO_2 biofixation rate (F_{CO_2} , % v/v), and minimal pH (pH_{min}) and maximal pH (pH_{max}) values obtained for the microalgal cultures

Carbon source	X_{max}	P_{max}	μ_{max}	F_{CO_2}	pH_{min}	pH_{max}
<i>Spirulina</i> sp. LEB 18						
NaHCO ₃	0.61±0.01 ^{aA}	0.04±0.01 ^{aA}	0.09±0.02 ^{aA}	–	10.0	10.8
Combustion gas	0.63±0.09 ^{aA}	0.06±0.01 ^{aA}	0.06±0.01 ^{aA}	20.5±0.05 ^{acA}	10.1	10.5
Carbon dioxide	0.59±0.04 ^{aA}	0.05±0.00 ^{aA}	0.11±0.01 ^{aA}	8.9±0.00 ^{aA}	9.5	10.8
NaHCO ₃ +40 ppm ashes	0.57±0.07 ^{aA}	0.04±0.01 ^{aA}	0.11±0.01 ^{aA}	–	10.0	10.8
Combustion gas	0.64±0.09 ^{aA}	0.06±0.03 ^{aA}	0.06±0.01 ^{aA}	11.6±0.03 ^{aA}	9.9	10.5
Carbon dioxide+40 ppm ashes	0.58±0.01 ^{aA}	0.05±0.01 ^{aA}	0.05±0.01 ^{aA}	12.2±0.01 ^{aA}	10.0	10.4
<i>Chlorella fusca</i> LEB 111						
Na ₂ CO ₃	0.70±0.04 ^{aB}	0.04±0.01 ^{aB}	0.08±0.02 ^{aB}	–	9.9	10.7
Combustion gas	0.55±0.09 ^{aB}	0.06±0.01 ^{aBC}	0.08±0.01 ^{aB}	36.8±0.03 ^{bB}	9.2	10.6
Carbon dioxide	0.84±0.02 ^{aB}	0.08±0.01 ^{aC}	0.12±0.02 ^{aB}	42.8±0.04 ^{bB}	8.9	10.0
Na ₂ CO ₃ +40 ppm ashes	0.76±0.03 ^{aB}	0.05±0.00 ^{aBC}	0.09±0.03 ^{aB}	–	9.7	10.4
Combustion gas	0.47±0.01 ^{aB}	0.06±0.01 ^{aBC}	0.09±0.07 ^{aB}	34.4±0.08 ^{bB}	8.2	10.4
Carbon dioxide+40 ppm ashes	0.66±0.10 ^{aB}	0.05±0.00 ^{aBC}	0.05±0.01 ^{aB}	29.5±0.01 ^{bcB}	8.4	9.4

Lowercase letters in the same column compare *Chlorella* and *Spirulina*. Uppercase letters in the same column compare *Spirulina* or *Chlorella* experiments only. Values denoted using the same letters in the same column indicate that the results did not differ significantly with a 99 % confidence level

by burning fossil fuels on such strains would be reduced compared with that on native microorganisms, eliminating the need to import tolerant strains.

Morais and Costa [30] isolated strains of *Scenedesmus obliquus* and *Chlorella kessleri* from a stabilization pond containing the liquid and solid effluents of the UTPM and found that they exhibited better growth when exposed to CO₂ concentrations higher than those in the emissions of thermoelectric plants. The absorption of atmospheric CO₂ by *Chlorella* has been studied as a method of bioremediation. This microalga exhibited a high capacity for CO₂ fixation and tolerance of this gas at concentrations above the output level of thermal power plants [30]. Furthermore, eukaryotic microorganisms have important differences in their cellular organization compared with those of prokaryotic microorganisms, which are related to their photosynthetic productivity. Chlorophyll molecules are not sequestered in chloroplasts in prokaryotic organisms (such as *Spirulina* sp. LEB 18) as they are in eukaryote organisms (such as *Chlorella fusca* LEB 111). The protection of chlorophyll molecules in eukaryotes renders them more resistant to destruction by the acidic compounds present in combustion gas [31].

The pH values of the cultures grown using combustion gas or CO₂ were approximately 8.2 and 10.8 before and after the injection of CO₂, respectively. According to Costa et al. [32], *Spirulina* requires an alkaline pH of between 8.3 and 11.0 and a high availability of carbonate or sodium bicarbonate for optimal growth. *Chlorella* microalga grow in media with a pH of between 6.5 and 7.5. Having been isolated from the ashes of stabilization ponds (pH 9.8) at the UTPM, the *Chlorella fusca* LEB 111 strain is alkaline resistant. An external carbon source that participates in the CO₂ ↔ HCO₃⁻ ↔ CO₃²⁻ chemical balance is generally required for microalgal growth. The pH value of the growth medium determines the distribution of these chemical species. The higher the pH, the more readily available is the CO₂ in the medium, which is converted into CO₃²⁻ [24, 33]. CO₂ is the carbon source used for the photosynthetic and autotrophic growth of microalgae.

In the experiments conducted using the solid residues, the pH of the media ranged from approximately 8.2 to 10.6, which caused no changes that would undermine growth. The ash obtained from burning coal can be acidic or alkaline. The sub-bituminous coal ash of the UTPM causes aqueous solutions to become alkaline (pH ≈ 9.8), a phenomenon that is associated with the presence of minerals such as calcite, amorphous silicates, hematite, quartz, oxides, and free carbon in this coal ash. The resultant level of alkalinity depends on the CaO content of the solution, and low pH values are due to the presence of condensed sulfuric acid in the ash particles. Over time, the excess alkali and alkaline earth oxides react with water and neutralize the acidity. Thus, the initially measured pH values may be low, but over time, neutralization occurs, causing an increased final pH value (Table 3) [34]. Thus, using ashes to culture microalgae can stimulate their CO₂ biofixation, maintaining favorable growth conditions at an alkaline pH, thus eliminating the need to add acids or bases to adjust the pH.

According to Radmann et al. [24], samples that were collected from effluent treatment ponds near the UTPM were highly alkaline (pH ≈ 10.0) due to the presence of ash derived from burning coal for thermal power generation. The pH of the medium is a factor that significantly affects the growth of algae. A change in the pH affects the solubility and availability of nutrients, the activity of enzymes, and the transport of substrates across the plasma membrane and of electrons in respiration and photosynthesis [30, 35].

The ashes derived from coal combustion consist mainly of oxides of silica (SiO₂), aluminum (Al₂O₃), iron (Fe₂O₃), and calcium (CaO) and trace metal elements (such as zinc, manganese, lead, chromium, nickel, copper, cadmium, and cobalt). The heavy metals that

pose environmental risks are the following, in descending order: lead, cadmium, mercury, chromium, cobalt, copper, and nickel [8]. Table 4 shows the content of heavy metals determined in the final microalgal biomasses.

The amount of nickel (Ni) that accumulated in the *Spirulina* sp. LEB 18 biomass could be attributed to the Ni content of the (Zarrouk) microalgal culture medium or to that of the coal ash. The nickel that accumulated in the *Chlorella fusca* LEB 111 biomass was due to that in the ash-decantation ponds and/or the addition of ashes to the culture medium because this metal is not present in BG11 medium. The cadmium (Cd) content of the samples was below the limit of detection (<0.03), indicating that less than $0.03 \mu\text{g L}^{-1}$ of the metal added to the culture or accumulated by *Chlorella fusca* LEB 111 was metabolized by this microalga (Table 4). Cu metal was present in the culture media of the microalga *Spirulina* sp. LEB 18 and *Chlorella fusca* LEB 111. The higher amount of Cu in the *Chlorella* biomass compared with that in the *Spirulina* biomass was also justified by this microalga having been exposed to the high metal concentrations near the UTPM.

Table 5 shows the concentration of Cu and Ni in the culture media ($\mu\text{g}_{\text{metal}} \text{L}^{-1}_{\text{medium}}$) and the concentrations determined in the final biomasses (grams of biomass produced per liter of medium). Table 5 shows that the biomass of *Spirulina* sp. LEB 18 grown using the combustion gas and ash had accumulated Cu metal at a concentration ($20.69 \mu\text{g L}^{-1}$) greater than that of the culture medium and of 40 ppm of ashes. This result may be explained by the presence of a higher concentration of ash due to the injection of combustion gas containing coal ashes. The higher concentration of Ni ($3.49 \mu\text{g L}^{-1}$) in this biomass compared with that of the microalgal culture medium can be attributed to the addition of 40 ppm of ash to the medium or the presence of ash in the combustion gas.

The higher concentration of Cu in the final biomass of *Chlorella fusca* LEB 111 compared with that of the microalgal culture medium was due to this strain being exposed to higher metal concentrations in the ashes of the decantation pond at the UTPM and consequent accumulation of Cu in the initial biomass culture. It was also observed that this microalga accumulated Cu to an average boundary of assimilation (45.44 ± 2.3) (Table 5).

According to the ANVISA decree no. 55,871 and decree no. 685 of 27 August 1998 and Resolution RDC no. 42 of 29 August 2013, the maximal tolerated levels of the inorganic contaminants Cd, Cu, and Ni are 1, 30, and $5 \mu\text{g g}^{-1}$, respectively. Microorganisms, including microalgae and cyanobacteria, can affect the speciation of metals due to their active or mediatory capacity in metal-mobilization or -immobilization processes, which affect the balance of metal species in the solid and liquid phases [36]. Cells, their excreted products, their cell walls, and their polysaccharides have the potential to absorb metals from the solutions

Table 4 Quantification of the heavy metal contents in the microalgal biomasses ($\mu\text{g g}^{-1}$)

Samples	Cd	Cu	Ni
<i>Spirulina</i> grown using NaHCO_3	<0.03	10.42	0.05
<i>Spirulina</i> grown using combustion gas and ashes	<0.03	17.96	3.03
<i>Chlorella</i> grown using Na_2CO_3	<0.03	37.38	2.19
<i>Chlorella</i> grown using ashes	<0.03	32.01	2.90
Ashes ^a	0.06	1.66	2.86

^a The ashes of coal combustion collected in phase C of the UTPM operation

Table 5 Concentrations of copper and nickel in the culture media and the final biomasses

Experiment	In the medium		In the biomass	
	Cu ($\mu\text{g L}^{-1}$)	Ni ($\mu\text{g L}^{-1}$)	Cu ($\mu\text{g L}^{-1}$)	Ni ($\mu\text{g L}^{-1}$)
<i>Spirulina</i> grown using NaHCO_3	20.10	10.01	11.44	0.06
<i>Spirulina</i> grown using combustion gas and ashes (40 ppm)	20.17	10.12	20.69	3.49
<i>Chlorella</i> grown using Na_2CO_3	20.10	–	47.09	–
<i>Chlorella</i> grown using ashes (40 ppm)	20.17	–	43.79	–

surrounding them, either in the form of precipitates or volatile substances. Microorganisms can modify the oxidation state of metals, allowing their detoxification [37].

Wang et al. [38] evaluated the potential of microalgal *Chlorella* sp. to eliminate metal ions from wastewater treatment plants and found that this microalgal species efficiently removed Al, Ca, Fe, Mg, and Mn metal ions. Bender and Cheung [39] used cyanobacteria to remove metals from water, reporting that the metal complexes bound to polysaccharides (of >200.000 Da). The leaching of ash from disposal areas may cause minor elements, such as heavy metals, to enter the water table, contaminating the current and potential sources of water and possibly entering the food chain via plants [40]. Thus, the use of ashes in the growth of microalgae can reduce the availability of toxic metals in the environment besides reducing the ash content with limited application and/or destination that cause severe industrial and environmental problems.

Conclusion

The maximum values of the concentration of biomass and biofixation of CO_2 were 0.84 g L^{-1} and 42.8 % (v/v), respectively, obtained from the growth of the microalga *Chlorella fusca* LEB 111 in the presence of 10 % (v/v) CO_2 . For *Spirulina* sp. LEB 18, the maximal values were 0.64 g L^{-1} and 20.5 %, respectively. These microalgae demonstrated their potential for using the gaseous and solid effluents of thermoelectric origin, presenting no significant differences in the values for the parameters of maximal biomass concentration and yield and the specific and daily CO_2 biofixation rates compared with those of cultures grown using carbon dioxide and bicarbonate and/or sodium carbonate. Therefore, these microalgae can be grown on a pilot scale for biofixation of the CO_2 in combustion gas, and the coal ashes can be used in their cultures, in which, in addition to minimizing environmental problems, the use of these alternative sources of CO_2 and minerals would reduce the cost of these nutrients, which represent a large fraction of the costs of producing these microorganisms.

Acknowledgments The authors would like to thank CNPq (National Council of Technological and Scientific Development) and CGTEE (Company of Thermal Generation of Electric Power) for their financial support of this study.

Conflict of Interests The authors declare that there is no conflict of interests regarding the publication of this paper.

References

1. Toledo-Cervantes, A., Morales, M., Novelo, E., & Revah, S. (2013). Carbon dioxide fixation and lipid storage by *Scenedesmus obtusiusculus*. *Bioresource Technology*, *130*, 652–658.
2. Eletrobras, CGTEE. Companhia de geração térmica de energia elétrica. Available from: www.cgtee.gov.br. Accessed 22 September 2015.
3. Liu, G., Vassilev, S. V., Gao, L., Zheng, L., & Peng, Z. (2005). Mineral and chemical composition and some trace element contents in coals and coal ashes from Huaibei coal field, China. *Energy Conversion and Management*, *46*, 2001–2009.
4. Bitjukova, L., Mõtlep, R., & Kirsimäe, K. (2010). Composition of oil shale ashes from pulverized firing and circulating fluidized-bed boiler in Narva thermal power plants, Estonia. *Oil Shale*, *27*, 339–353.
5. Chiu, S. Y., Kao, C. Y., Chen, C. H., Kuan, T. C., Ong, S. C., & Lin, C. S. (2008). Reduction of CO₂ by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. *Bioresource Technology*, *99*, 3389–3396.
6. Chiu, S. Y., Kao, C. Y., Tsai, M. T., Ong, S. C., Chen, C. H., & Lin, C. S. (2009). Lipid accumulation and CO₂ utilization of *Nannochloropsis oculata* in response to CO₂ aeration. *Bioresource Technology*, *100*, 833–838.
7. Rosa, A. P. C., Carvalho, L. F., Goldbeck, L., & Costa, J. A. V. (2011). Carbon dioxide fixation by microalgae cultivated in open bioreactors. *Energy Conversion and Management*, *52*, 3071–3073.
8. Flues, M., Sato, I. M., Scapin, M. A., Cotrim, M. E. B., & Camargo, I. M. C. (2013). Toxic elements mobility in coal and ashes of Figueira coal power plant, Brazil. *Fuel*, *103*, 430–436.
9. Ctvrtnickova, T., Mateo, M. P., Yañez, A., & Nicolas, G. (2009). Characterization of coal fly ash components by laser-induced breakdown spectroscopy. *Spectrochimica Acta Part B: Atomic Spectroscopy*, *64*, 1093–1097.
10. Antelo, F. S., Anschau, A., Costa, J. A. V., & Kalil, S. J. (2010). Extraction and purification of C-phycoyanin from *Spirulina platensis* in conventional and integrated aqueous two-phase systems. *Journal of the Brazilian Chemical Society*, *21*, 921–926.
11. Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, *25*, 294–306.
12. Haase, S. M., Huchzermeyer, B., & Rath, T. (2012). PHB accumulation in *Nostoc muscorum* under different carbon stress situations. *Journal of Applied Phycology*, *24*, 157–162.
13. Morais, M. G., Costa, J. A. V., Marins, L. F. F., Reichert, C. C., Dalcanton, F., & Durante, A. J. (2008). Isolation and characterization of a new *Arthrospira* strain. *Zeitschrift für Naturforschung Section C*, *63c*, 144–150.
14. Zarrouk, C. (1966). Contribution a l'étude cyanophyceae, influence de divers facteurs physiques et chimiques sur la croissance et photosynthese de *Spirulina maxima* Geitler. University of Paris.
15. Rippka, R., Deruelles, J., Waterbury, J. W., Herdman, M., & Stanier, R. G. (1979). Genetic assignments, strain histories and properties of pure cultures of Cyanobacteria. *Journal of General Microbiology*, *111*, 1–61.
16. Morais, M. G., & Costa, J. A. V. (2007). Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. *Journal of Biotechnology*, *129*, 439–445.
17. Reichert, C. C., Reinehr, C. O., & Costa, J. A. V. (2006). Semicontinuous cultivation of the cyanobacterium *Spirulina platensis* in a closed photobioreactor. *Brazilian Journal of Chemical Engineering*, *23*, 23–28.
18. Radmann, E. M., Reinehr, C. O., & Costa, J. A. V. (2007). Optimization of the repeated batch cultivation of microalga *Spirulina platensis* in open raceway ponds. *Aquaculture*, *265*, 118–126.
19. Baumgarten, M. G. Z., Wallner-Kersanach, M., & Niencheski, L. F. H. (2010). *Manual de análises em oceanografia química* (2nd ed.). Rio Grande: FURG. 172p.
20. Schmidell, W., Lima, A. U., Aquarone, E., & Borzani, W. (2001). *Biotecnologia industrial*. São Paulo: Edgard Blücher LTDA.
21. Bailey, J. E., & Ollis, D. F. (1986). *Biochemical engineering fundamentals* (2nd ed.). Singapore: McGraw-Hill.
22. Binaghi, L. D., Borghi, A., Lodi, A., Converti, A. D., & Borghi, M. (2003). Batch and fed-batch uptake of carbon dioxide by *Spirulina platensis*. *Process Biochemistry*, *38*, 1341–1346.
23. Basu, S., Roy, A. S., Mohanty, K., & Ghoshal, A. K. (2013). Enhanced CO₂ sequestration by a novel microalga: *Scenedesmus obliquus* SA1 isolated from bio-diversity hotspot region of Assam, India. *Bioresource Technology*, *143*, 369–377.
24. Radmann, E. M., Camerini, F. V., Santos, T. D., & Costa, J. A. V. (2011). Isolation and application of SO_x and NO_x resistant microalgae in biofixation of CO₂ from thermoelectricity plants. *Energy Conversion and Management*, *52*, 3132–3136.

25. Pires, J. C. M., Alvim-Ferraz, M. C. M., Martins, F. G., & Simões, M. (2012). Carbon dioxide capture from flue gases using microalgae: engineering aspects and biorefinery concept. *Renew and Sustainable Energy Reviews*, *16*, 3043–3053.
26. Brown, L. M. (1996). Uptake of carbon dioxide from flue gas by microalgae. *Energy Conversion and Management*, *37*, 1363–1367.
27. Chu, W. L., Phang, S. M., & Goh, S. H. (1996). Environmental effects on growth and biochemical composition of *Nitzschia inconspicua* Grunow. *Journal of Applied Phycology*, *8*, 389–396.
28. Renaud, S. M., Thinh, L. V., Lambridis, G., & Parry, D. L. (2002). Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture*, *211*, 195–214.
29. Salih, F. M. (2011). Microalgae tolerance to high concentrations of carbon dioxide: a review. *Journal of Environmental Protection*, *2*, 648–654.
30. Morais, M. G., & Costa, J. A. V. (2007). Isolation and selection of microalgae from coal fired thermolectric power plant for biofixation of carbon dioxide. *Energy Conversion and Management*, *48*, 2169–2173.
31. Tastan, B. E., Duygu, E., Atakol, O., & Donmez, G. (2012). SO₂ and NO₂ tolerance of microalgae with the help of some growth stimulators. *Energy Conversion and Management*, *64*, 28–34.
32. Costa, J. A. V., Colla, L. M., Filho, P. D., Kabke, K., & Weber, A. (2002). Modelling of *Spirulina platensis* growth in fresh water using response surface methodology. *World Journal of Microbiology and Biotechnology*, *18*, 603–607.
33. Vonshak, A. (1997). *Spirulina platensis (Arthrospira): physiology, cell-biology and biotechnology*. London: Taylor & Francis.
34. Soares, E. R., Mello, J. W. V., Schaefer, E. G. R., & Costa, L. M. (2006). Cinza e carbonato de cálcio na mitigação de drenagem ácida em estéril de mineração de carvão. *Revista Brasileira de Ciência do Solo*, *30*, 171–181.
35. Sankar, V., Daniel, D. K., & Krastanov, A. (2011). Carbon dioxide fixation by *Chlorella minutissima* batch cultures in a stirred tank bioreactor. *Biotechnology & Biotechnological Equipment*, *25*, 2468–2476.
36. Gadd, G. M. (2004). Microbial influence on metal mobility and application for bioremediation. *Geoderma*, *122*, 109–119.
37. Singh, P., & Cameotra, S. S. (2004). Enhancement of metal bioremediation by use of microbial surfactants. *Biochemical and Biophysical Research Communications*, *319*, 291–297.
38. Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., & Liu, Y. (2010). Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry and Biotechnology*, *162*, 1174–1186.
39. Bender, J., Rodriguez-Eaton, S., Ekanemesang, U. M., & Phillips, P. (1994). Characterization of metal-binding bioflocculants produced by the cyanobacterial component of mixed microbial mats. *Applied and Environmental Microbiology*, *60*, 2311–2315.
40. Fungaro, D. A., & Silva, M. G. (2002). Utilização de zeólita preparada a partir de cinzas residuárias de carvão como adsorvedor de metais em água. *Quim Nova*, *6*, 1081–1085.