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A N I N T E R N A T I O N A L J O U R N A L

Use of Solid Waste from Thermoelectric Plants for the Cultivation of Microalgae

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ABSTRACT

The aim of this study was to analyze the influence of solid waste on the cultivation of the microalgae Spirulina *sp. LEB 18 and* Chlorella fusca *LEB 111 with 0, 40, 80 and 120 ppm of mineral coal ash. The addition of the ash did not inhibit the cultivation of microalgae at the tested concentrations, showing that it could be used for the cultivation of these microalgae due to the minerals present in the ash, which might substitute the nutrients needed for their growth.*

Key words: ashes, *Chlorella fusca* LEB 111, microalgae, mineral coal, *Spirulina* sp. LEB 18.

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INTRODUCTION

As technology and industry develops, there is increasing environmental concern about the release of industrial waste, which can damage the ecosystem. The rapid expansion of different industrial sectors has resulted in increased amounts of toxic wastes. Mineral coal ash is one of the most produced solid wastes in Brazil in terms of volume (Soares et al. 2008). The burning of coal from south Brazil produces a high percentage of ash, around 53% (w/w), which is a serious environmental problem. Due to the presence of potentially toxic trace elements in this waste, it must be disposed of and used carefully, and an appropriate site for disposal must be chosen, in order to protect the ground and the surface water (Benito et al 2001; Soares et al. 2008).

Generally, ashes are deposited in open pit mines, or in the landfills near the plant (Depoi et al. 2008). Less than 30% of ash is reused in the construction industry; the rest is improperly dumped in landfills, which can harm human health and the environment due to the leaching of toxic metal ions present in the ash (Soares et al. 2008). Metal ions, even at low concentrations, can become toxic to living beings (Burgstaller and Schinner 1993). Therefore, it is essential to find methods that reduce the levels of contamination caused by these metals. One alternative to combat this type of pollution is the use of biological processes (Schmitz et al. 2012).

The use of microalgae is one such biological process with economic, nutritional and ecological importance due to a microalga's capacity to double its biomass within 24 h (Becker 2004; Antelo et al. 2010). Microalgae are capable of removing heavy metals from the environment due to their active, or mediating ability in mobilization, or immobilization processes that influence the equilibrium of metallic species between the solid and liquid phases. Mobilization is the process through which an initial insoluble state, corresponding to a solid phase, turns into a final soluble state in aqueous phase. Immobilization is the process through which an initial soluble state in aqueous phase turns into a final insoluble state in solid phase (Gadd 2004).

The objective of this study was to analyze the influence of solid wastes at different concentrations on the cultivation of the microalgae, *Spirulina* sp. LEB 18 and *Chlorella fusca* LEB 111.

MATERIAL AND METHODS

Microorganisms and culture media

The microalgae used in this study were *Spirulina* sp. LEB 18, isolated from Mangueira lagoon, latitude 33º31'08''S and longitude 53º22'05''W. *Chlorella fusca* LEB 111 was isolated from the pond for stabilization of effluents and waste at the President Medici Thermoelectric Power Plant (UTPM), latitude 24°36'13''S and longitude 52º32'43''W (Candiota RS); (Morais et al. 2008).

Spirulina sp. LEB 18 was cultivated in Zarrouk medium with the following composition (g L^{-1}): $NaHCO_3$ (16.8); K_2HPO_4 (0.50); $NaNO_3$ (2.50); K_2SO_4 (1.00); NaCl (1.00); $MgSO_4.7H_2O$ (0.20); $CaCl_2$ (0.04); $FeSO_4.7H_2O$ (0.01); EDTA (0.08); H_3BO_3 (2.86); $MnCl_2.4H_2O$ (1.81); $ZnSO_4.7H_2O$ (0.222); $CuSO_4.5H_2O$ (0.079); Na_2MoO_4 (0.015); in (mg L^{-1}), NH_4VO_3 (22.86); $K_2Cr_2(SO_4)_4.24H_2O$ (96); $NiSO_4.7H_2O$ (47.85); $Na_2WO_4.2H_2O$ (17.94); $TiOSO_4.H_2SO_4.8H_2O$ (61.1); $Co(NO_3)_2.6H_2O$ (43.98) (Zarrouk 1966).

Chlorella fusca LEB 111 was grown in BG11 medium with the following composition (g L^{-1}): $NaNO_3$ (1.50); $K_2HPO_4.3H_2O$ (0.04); $MgSO_4.7H_2O$ (0.075); $CaCl_2.2H_2O$ (0.036); ammonium ferric citrate (0.006); EDTA (0.001); Na_2CO_3 (0.02); citric acid (0.006); $ZnSO_4.7H_2O$ (0.222); $MnCl_2.4H_2O$ (1.81); $Na_2MoO_4.2H_2O$ (0.390); H_3BO_3 (2.86); $CuSO_4.5H_2O$ (0.079); $Co(NO_3)_2.6H_2O$ (0.0494) (Rippka 1979).

Cultivation conditions

The cultivations were carried out in discontinuous mode in 2 L Erlenmeyer type closed photobioreactors, with a working volume of 1.8 L (replacement of evaporation with sterile distilled water) and initial microalgal concentration of 0.2 g L^{-1}. The temperature was maintained at 30°C in a thermostated chamber with a photoperiod of 12 h light/dark (Reichert et al. 2006) and 43.2 µmol m^{-2} s^{-1} illuminance provided by fluorescent lamps (General Electric) (Morais and Costa 2007).

The cultures were stirred using sterile compressed air. The solid wastes (coal fly ash) were provided by UTPM (Table 1), collected from Silo 1 (Phase C) and added to the culture medium of each microalga at concentrations of 0, 40, 80 and 120 ppm. The ash concentrations were selected according to the emission standard, established in UTPM's environmental licensing process. The experiments were carried out in duplicate and lasted 12 d.

Table 1 - Chemical composition of the utilized coal fly ash

Components	Composition
Major elements (%)	
SiO_2	65,8
Al_2O_3	21,5
Fe_2O_3	4,6
TiO_2	0,7
P_2O_5	<0,03
CaO	1,8
MgO	0,8
Na_2O	0,1
K_2O	1,9
SO_3	1,4
loss on ignition	1,4
Trace elements (mg kg^{-1})	
Co	18,0
Cr	68,8
Cu	31,5
Mn	183,0
Ni	12,5
Zn	103,8

Font: UTPM.

Analytical determinations

The biomass concentration was measured daily by reading the optical density at 670 nm in a spectrophotometer (QUIMIS Q798DRM), with a calibration curve that related optical density to the dry biomass weight for each microalga (Radmann et al. 2007; Rosa et al. 2011). The pH of the cultures was measured using a digital pH meter every 24 h (LUTRON PH-221).The concentration of carbon, hydrogen and nitrogen in the final biomass was measured for each experiment by using the elemental analyzer CHNS/O (Perkin-Elmer 2400, USA). The acetanilide certificate standard was used to calibrate the equipment (Perkin Elmer, USA).

Kinetic parameters

The biomass concentration values were used to determine the maximum specific growth rates ($\mu_{máx,}$ d^{-1}), maximum cell concentrations ($X_{máx,}$ g L^{-1}) and maximum yields ($P_{máx,}$ g L^{-1} d^{-1}). The maximum yield was obtained according to $P = (X_t - X_0)\ (t - t_0)^{-1}$, where X_t was the biomass concentration (g L^{-1}) at time t (d), and X_0 the biomass concentration (g L^{-1}) at time t_0 (d) (Schmidell et al. 2001). The maximum specific growth rate ($\mu_{máx,}$ d^{-1}) was obtained by exponential regression applied to the logarithmic phase (Bailey and Ollis 1986).

Statistical analysis

The experimental results were evaluated using the analysis of variance (ANOVA) and Tukey's test to compare the means of the kinetic parameters, with a significance level of 99% ($p \leq 0.01$). Thus, it was possible to analyze the influence of residues in the microalgal cultures.

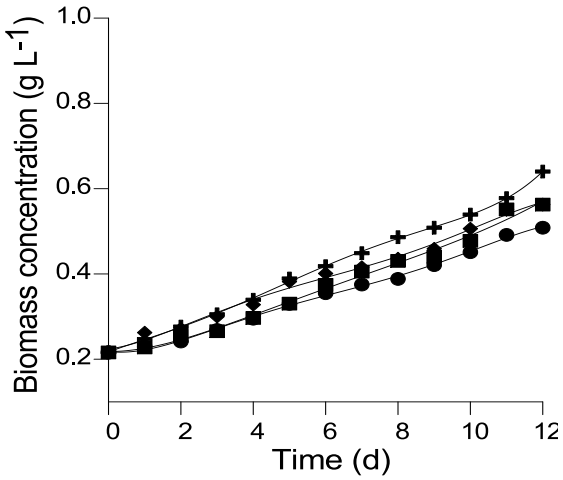
RESULTS ANS DISCUSSION

The maximum biomass concentration (0.64 g L^{-1}) was obtained for the *Spirulina* sp. LEB 18 cultivated with 120 ppm ash. The kinetic parameters of growth, maximum productivity (0.04 g L^{-1} d^{-1}) and maximum specific growth rate (0.10 d^{-1}) were also maximum in the experiment with higher ash content (Table 2). The growth curves of *Spirulina* sp. LEB 18 and *C. fusca* LEB 111 (Figs. 1 a and b) did not present a *"lag"*, or latency phase. This phase occurs in many cultures after the inoculation of the microalga in the culture medium, with no reproduction, because the cells synthesizeenzymes required for the cultures in different conditions than those under which the initial inoculum is maintained. The duration of this phase depends on the initial inoculum concentration, the pre-cultivation time and the physiological state of the microorganism. Native species of microalgae are more tolerant of local conditions and exhibit higher rates of photosynthesis and biomass production (Salih 2011). The environmental impact of the high concentrations of solid residues produced by burning the fossil fuels on such strains would be reduced compared with that on native

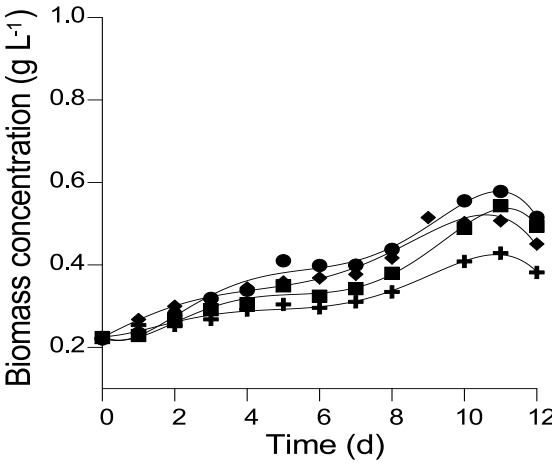
microorganisms, eliminating the need to look for tolerant strains.

The microalgae grew immediately after inoculation (transition phase), and the onset of cell reproduction was observed in the initial phase of the experiment (Figs. 1a and 1b). There was no lag phase, despite the addition of a new factor (ash), because many of the minerals contained in the ashes (cobalt, chromium, copper, manganese, molybdenum, nickel, zinc), were also used by the culture in which the inoculum was maintained (Depoi et al. 2008). The linear phase is characteristic of the constant reproduction velocity. This behavior suggests that there are certain limitations in the transport of nutrients to the microorganism-medium interface, with respect to oxygen dissolved in the culture (Schmidell et al. 2001). The cultures might have been adversely affected and/or had a sharp linear phase, due to the influence of dissolving CO_2, because the reactors used did not enable much time for the gas to reside in the culture. The use of tubular reactors might reduce this problem.

In the growth curves of *Spirulina* sp. LEB 18 (Fig. 1a), a stationary phase followed by a decline and/or cell death was not observed until the end of cultivation (12 d). For *C. fusca* LEB 111 (Fig. 1b), the cell death phase was observed at 12 d of culture for all of the experiments (Fig. 1b), which might have occurred due to the limitation of the BG11 medium's carbon source. The *Spirulina* sp. LEB 18 had optimal growth (maximum) when cultivated with 120 ppm of ash. *C. fusca* LEB 111 did not present a maximum concentration of biomass when grown with the highest amount of ash, and there was no statistically significant difference ($p < 0.01$) from the other experiments (Table 2).



(a)



(b)

Figure 1- Growth curves of the microalgae *Spirulina* sp. LEB 18 (a) and *Chlorella fusca* LEB 111 (b) cultivated (●) 0 (■) 40 (♦) 80 e (+) 120 ppm ash

Table 2 - Maximum biomass concentration (X_{max}), maximum productivity (P_{max}) and maximum specific growth rate (μ_{max}) obtained for the microalgae *Spirulina* sp. LEB 18 and *Chlorella fusca* LEB 111cultivated with different concentrations of ash (ppm)

Ash concentration (ppm)	**$X_{máx}$ (g L^{-1})**	**$P_{máx}$ (g $L^{-1}d^{-1}$)**	**$\mu_{máx}$ (d^{-1})**
Spirulina sp. LEB 18			
0	0.51±0.03[a]	0.03±0[a]	0.07±0[a]
40	0.56±0.09[a]	0.03±0[a]	0.08±0.01[a]
80	0.56±0.03[a]	0.03±0[a]	0.07±0[a]
120	0.64±0.01[a]	0.04±0[a]	0.10±0.01[a]
Chlorella fusca LEB 111			
0	0.58±0.06[a]	0.03±0.01[a]	0.09±0.01[a]
40	0.54±0.05[a]	0.03±0.01[a]	0.08±0.01[a]
80	0.52±0.05[a]	0.03±0[a]	0.07±0.01[a]
120	0.43±0.01[a]	0.02±0[a]	0.05±0.01[b]

Same letters in the same column, the results are not statistically different at a 99% level of confidence.

The pH of the cultures with *Spirulina* sp. LEB 18 and *C. fusca* LEB 111 varied from 9.3 to 10.3. Figure 2a showed that the pH (alkaline) remained similar for all of the experiments of *Spirulina* sp. LEB 18 throughout the 12 d of culture. Figure 2b showed that *C. fusca* LEB 111 medium without ash had a higher pH. Low pH values in the culture with 120 ppm ash mighthave limited the form of inorganic carbon available in the culture medium (BG11) of *C. fusca* LEB 111. The higher the medium's pH, the more easily bicarbonate is converted into carbonate, which is the standard carbon source in BG11 medium. Thus, the maximum biomass concentration (0.58 g L^{-1}) was obtained in the culture with the highest pH (Fig. 2b), with no statistical difference between the experiments with the addition of ash for *C. fusca.* According to Lourenço (2006), controlling the pH is essential so that components of the culture medium can be effectively absorbed, directly affecting the availability of various chemical elements.

The cultivation of *Spirulina* requires an alkaline medium (pH 8.3 to 11.0) with high availability of carbonate, or bicarbonate as optimal growth conditions (Costa et al. 2002). This suggested that the pH did not impair the growth in the *Spirulina* cultures (Fig. 2a). The microalga *Chlorella* kept growing at neutral pH (6.5 to 7.5). The fact that *C. fusca* LEB 111 was isolated from UTPM's ash decantation ponds, justified its resistance to alkalinity. The pH is directly related to the form of inorganic carbon available in the culture, because alkaline media favor the bicarbonate form (HCO_3^-), which is more easily assimilated than CO_2, and accounts for more than 80% when the pH ranges from 7.0 to 9.0. The free CO_2 and carbonate (CO_3^{-2}) forms predominate below, or above these values, respectively. Very low pH values (<5.0) can inhibit microalgal growth, leading to unavailability of nutrients (Kirk 1994).

The UTPM ash is mainly made up of silica (SiO_2), alumina (Al_2O_3), iron oxide (Fe_2O_3) and calcium oxide (CaO). The sub-bituminous coal ash from Candiota produces alkaline solutions when in contact with water, and this phenomenon is associated with the existence of minerals, such as calcite, amorphous silicates, hematite, quartz, oxides and free carbon. The

alkalinity depends on the CaO content, and the low pH values are due to the presence of condensed sulfuric acid in the ash particles. Over the time, the excess alkali and alkaline-earth oxides react and neutralize the acidity (Flues et al. 2013). Thus, initial pH measurements could present low values, but over the time, the final rise in pH occurred as was observed in the cultures with the studied microalgae (Fig. 2).

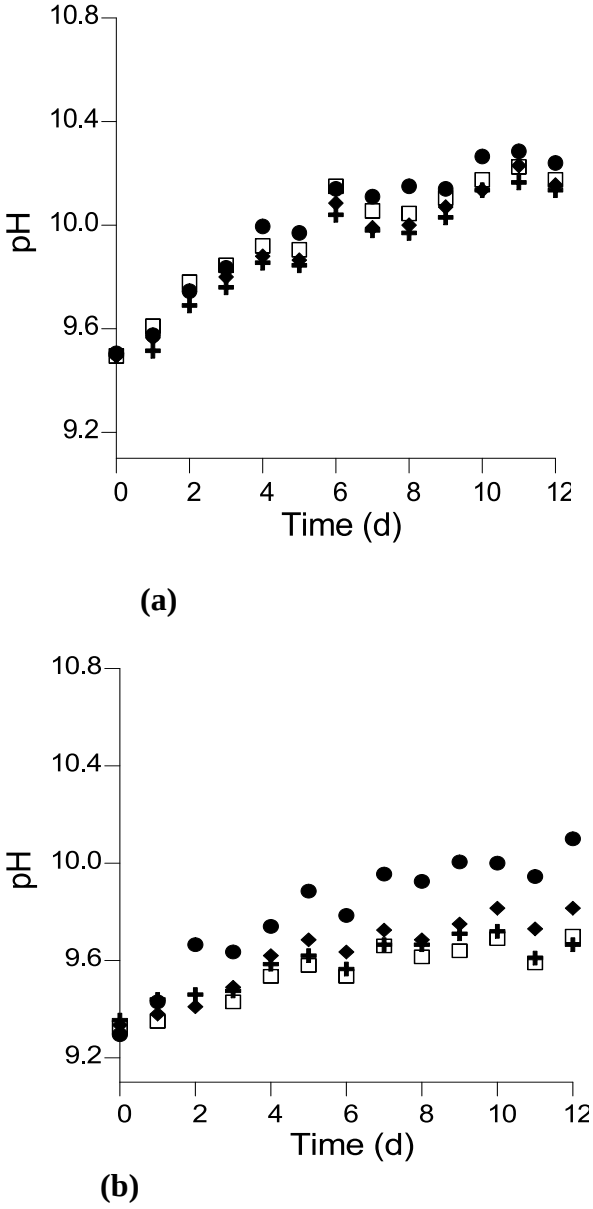


Figure 2- pH curves for the experiments with the microalgae *Spirulina* sp. LEB 18 (a) and *Chlorella fusca* LEB 111 (b) cultivated with (●) 0 (+) 40 (□) 80 e (♦) 120 ppm of ash

The solubility of the minerals varies according to the particle size. The UTPM ash that is emitted from coal combustion (chimney outlet) has a mean diameter of 59.82 µm (Eletrobras/ CGTEE 2013). According to Pires and Queirol (2004), some of the ash's elements are more soluble (>40%) when the particle size is smaller (arsenic, cobalt, strontium, barium, zinc and nickel). The same behavior was observed for zirconium, chromium, vanadium, hafnium, and thallium, but with low solubility (5%). On the other hand, iron, niobium, cesium, lithium and tungsten had poor solubility for all fraction sizes. Sulfur and molybdenum have different solubility profiles, and are more soluble than the other elements. Thus, the large amount of minerals in the ash, dissolved in the culture medium, can partly replace the essential nutrients required to cultivate microalgae, such as Fe (Pane et al. 2008; Ctvrtnickova et al. 2009). Certain elements are essential for the growth and composition of microalgae. Carbon, nitrogen, hydrogen, oxygen, phosphorous, magnesium, copper, zinc, sulfur, potassium, calcium and molybdenum are considered necessary for all algae. Sodium, cobalt, vanadium, selenium, silicon, chlorine, iodine and boron are required only for some algae. The different nutrient elements are required in varying concentrations. Depending on the amount required for the metabolic processes, the nutrient elements can be classified into two fundamental categories: macronutrients (C, H, O, N, P, S, K, Mg, Si, Fe) and micronutrients (Mn, Cu, Zn , Mo, V, B, Co, Ca, In, Se, Ni) (Lourenço 2006).

In addition to accumulating minerals, microalgae are able to accumulate toxic metals, such as cadmium (Solisio et al. 2008). Several studies with the microalgae *Chlorella* and *Spirulina* have been carried out to remove toxic heavy metals from waste water (Lodi et al. 2008; Pane et al. 2008; Solisio et al. 2008). The microbial biochemical mechanism does not degrade the contaminant atom, but changes the toxic metal's oxidation state, thus enabling its detoxification. Therefore, these microorganisms have the ability to concentrate, or remove metals, whether in the form of precipitates, or volatile substances, turning the species into less toxic and more readily available compounds (Singh and Cameotra 2004).

CONCLUSION

The maximum biomass concentration (0.64 g L^{-1}) was obtained in the cultivation of *Spirulina* sp. LEB 18 grown with 120 ppm of ash, compared with *C. fusca* LEB 111 (0.58 g L^{-1}), with no significant difference from the standard growth medium of both microalgae. The results showed that the solid waste originating from the burning of coal did not hamper the growth of microalgae at the tested concentrations. These residues may replace part of the metals that are essential for the growth of microalgae, and these may also enable the decontamination of toxic metals by changing the oxidation state of these elements. In these processes, the microbial metabolism removes pollutants, turning it into raw materials for the generation of biomass and bioproducts that can be used for energy, biofuels and biopolymers. The costs of the culture medium can be significantly reduced by replacing the nutrients contained in the medium by low cost and difficult to dispose of alternative sources, such as ash. Thus, the biological processes combine to reduce the environmental pollution with the production of bioproducts with reduced costs.

CONFLICT OF INTERESTS

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

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