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Growth performance of an acidophilic microalga cultivated in treated wastewater

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Abstract

Microalgae cultivation has been applied in Environmental Biotechnology as a potentially efficient option for CO_2 biofixation and biofuel production. However, it is important to develop low cost strategies for microalgae production. A possibility is the use of wastewater as an alternative culture medium. This study evaluated the growth performance of an acidophilic strain of microalga - *Chlamydomonas acidophila* LAFIC-004 - cultivated in wastewater. Three experimental treatments were evaluated. The first used synthetic culture medium (T1), in the second, 50% of wastewater was added to the synthetic medium (T2) and in the third, 100% wastewater was used as culture medium (T3). Growth performance was evaluated by measuring cell density and dry biomass along the culturing period. T1 and T2 generated higher productivities (0.13 ± 0.02 and 0.10 ± 0.01 g L^{-1} day^{-1}, respectively) with no significant differences between them. Although T3 treatment showed lower productivity (0.08 ± 0.01 g L^{-1} day^{-1}), the results indicated that the wastewater can be used the sole culture medium or in combination with synthetic media for growing *C. acidophila* LAFIC-004.

Key Words: extremophilic microalgae; biomass production; *Chlamydomonas acidophila*; culture medium; wastewater.

Introduction

Microalgae have been increasingly cultivated around the world for purposes such as live feed to aquaculture species, human food supplement (nutraceuticals) and as a source of special proteins, lipids, pigments and antioxidants (Richmond 2004). Microalgae cultivation has been also applied in environmental biotechnology as a potentially efficient option for CO_2 biofixation from atmospheric emissions and for biofuel production (Chisti 2007, Morais and Costa 2007, 2008, Chiu et al. 2008, 2009, Yoo et al. 2010, Jiang et al. 2011, Tang et al. 2011, Ho et al. 2012). However, economic viability is still hampered by the high cost involved in the production process. One of the alternatives to reduce the production cost is using wastewater as a source of nutrients, having as an additional benefit the absorption of nutrients that have polluting potential (Kumar et al., 2010). To achieve this, it is necessary to select algae that present satisfactory growth in the wastewater.

In addition, the injection of CO_2 from atmospheric emissions has the potential to increase algal growth at low cost, promoting biofixation of this greenhouse gas. However, injecting CO_2 in the cultures may lead to culture media acidification (Babcock et al. 2002). Thus, it is important to monitor and control pH in order not to adversely affect microalgae growth performance. Nevertheless, acidification of the culture medium may be an advantage to microalgae species that are adapted to low pH (acidophilic microalgae), because it reduces the incidence of competing and predator organisms (Andersen 2005).

The aim of this study was to evaluate the influence of domestic wastewater, used as alternative culture medium, on the growth performance of an acidophilic microalgal strain.

Materials and Methods

Microalgae strain and culture conditions

The strain of microalga used in this research was isolated from acid mine drainage (AMD) of the coal mining region of Santa Catarina State in southern Brazil (Lat. 28°35'S, Long. 49°27'W) and held in the Laboratory of Phycology (LAFIC) at the Federal University of Santa Catarina (UFSC) as *Clamydomonas acidophila* LAFIC-004. The strain is maintained in Modified Acid Medium (MAM, Olaveson and Stokes,

1989), pH 3.6, irradiance of 50 µmol.m^{-2}.s^{-1} (fluorescent lamps), photoperiod of 12 h and temperature of 22 ± 2 °C.

Experimental design

The growth performance of *C. acidophila* LAFIC-004 was evaluated in a growth experiment with 3 different treatments. In the treatment 1 (T1), considered as control, the strain was cultured in TAP medium (93 mg L^{-1} $N\text{-}NH_4^+$, 81.7 mg L^{-1} $P\text{-}PO_4^{3-}$) according to Gorman and Levine (1965), which is indicated for cultivation of *Chlamydomonas* spp. Treatment 2 (T2) consisted of a mixture of 50% TAP medium and 50% wastewater, and treatment 3 (T3) was 100% wastewater. Before the experimental evaluation the strain was acclimated to the conditions of the 3 treatments for 10 days. Each treatment consisted of four replicates, randomly distributed, totaling 12 experimental units (2,000 mL Erlenmeyer with 1,000 mL of culture volume). The inoculation was done with culture in exponential growth phase, generating an initial dry biomass of 0.13 ± 0.02 g L^{-1} in each flask. The experimental ambient conditions were 25 ± 1 ^oC, constant aeration, with a flow of 0.3 L min^{-1} of atmospheric air enriched with CO_2 (1% vv^{-1}) and 24h of light with an irradiance of 150 µmol m^{-2} s^{-1}, provided by daylight fluorescent lamps (80W). The experimental time was ten days. The pH and turbidity of the experimental units were monitored daily.

Wastewater characteristics

The wastewater used as culture medium consisted of treated domestic effluent from a plant composed by an up-flow anaerobic sludge blanket (UASB), followed by an activated sludge system and a final disinfection with sodium hypochlorite. The plant operates with an average flow of 50 L s^{-1} serving a population of 36,000 inhabitants. The wastewater quality parameters are shown in Table 1. The values represent averages of two samplings performed immediately prior to the collection for the experiment.

Table 1. Water quality parameters of the wastewater used as alternative culture medium. Data provided by the system administrator company.

Parameter	Mean values ± standard deviation(n= 2)
Temperature (^oC)	26.1 ± 0.3
BOD (mg L^{-1})	28.0 ± 4.2
QOD (mg L^{-1})	43.7 ± 6.5
Total Phosphorus (mgP L^{-1})	6.10 ± 2.3
Suspended Solids (mg L^{-1})	19.5 ± 7.8
Ammonia-NH_4 (mg L^{-1})	34.2 ± 0.4
Nitrite-NO_2^- (mg L^{-1})	0.5 ± 0.1
Nitrate-NO_3^- (mg L^{-1})	2.3 ± 0.5
Total Nitrogen (mg L^{-1})	36.0 ± 5.8
Total Alkalinity (mg $CaCO_3$ L^{-1})	193.4 ± 11.7
pH	7.4 ± 0.1

The wastewater samples were chlorinated 24 hours prior to the experiment and immediately prior to inoculation the chlorine was neutralized with sodium thiosulfate.

Growth parameters

For the determination of growth parameters, aliquots of each experimental unit were collected daily for the quantification of cells using a Neubauer chamber. The specific growth rate (μ, day^{-1}) was calculated as $\mu=(LnX_t-LnX_0)/(t-t_0)$, where X_0 is the initial cell density (cells mL^{-1}) at time t_0 (day) and X_t is the maximum cell density (cells mL^{-1}) at time t (day).

Dry biomass was determined daily by gravimetry (Arredondo-Vega and Voltolina, 2007), after filtering known sample volumes through pre-dried glass fiber filters (0.45 μm pore size). The productivity (g L^{-1} day^{-1}) was calculated as dry biomass as a function of time and biomass accumulation (g L^{-1}) was calculated by the difference between final and initial dry biomass.

Statistical analysis

The results were submitted to analysis of variance (ANOVA, $p < 0.05$). The Tukey's test was applied to compare the mean values of experimental treatments when significant differences were detected. Results of turbidity and dry biomass were correlated and compared by linear regression analysis (Zar, 1996).

Results and Discussion

Growth performance

Cell density values throughout the cultivation period are shown in Figure 1a. Treatments T2 and T3 (50% and 100% wastewater, respectively) presented the highest cell densities, with no significant difference between them ($p<0.05$). Both values were significantly greater than the control (T1) after the seventh day of culture.

Data describing the growth and yield of the strain showed similar values among treatments (Table 2). If on the one hand this does not necessarily show a better performance of the strain in the wastewater media, on the other hand, it shows that the strain can be cultivated in 100% wastewater, not being necessary to use a synthetic medium. This data indicates robustness of the strain and suggests the possibility of cultivation at low cost, with possible additional benefit in bioremediation on nutrients of the wastewater.

Tang et al. (2011), using a synthetic culture medium, with a temperature of 25°C and pH 7, without CO_2 addition, reported a specific growth rate (μ) of 0.5 and 0.6 day^{-1} when cultivating *Scenedesmus obliquus* and *Chlorella pyrenoidosa*, respectively. Already with the addition of 5% (v v^{-1}) of CO_2 in the cultures, both present a specific growth rate of 0.9 day^{-1}. Radmann et al. (2011) cultivated *S. obliquus* and *Chlorella vulgaris* isolated from effluent treatment lagoons with high alkalinity, these microalgae presented better growth results when cultivated with the addition of 12% (v v^{-1}) of CO_2 at a temperature of 30 °C and pH between 8 to 9. Both had specific growth rates of 0.2 day^{-1}. Thus, it is possible that the strain of *Chlamydomonas acidophila* cultivated in this work can grow more efficiently with the increase of CO_2 concentration introduced into the photobioreactors.

Table 2. Values of Maximum Cell Density (MDC), Specific Growth Rate (μ) and Productivity reached in different culture media tested.

Growth Parameter	T1 (TAP Medium)	T2 (50%Wastewater)	T3 (100%Wastewater)
MCD (10^4 cells mL^{-1})	819 ± 230 a	1,136 ± 122 b	1,151 ± 80 b
Specific growth rate (μ) (day^{-1})	0.75 ± 0.11 a	0.73 ± 0.08 a	0.80 ± 0.14 a
Productivity (g L^{-1} day^{-1})	0.13 ± 0.02 a	0.10 ± 0.01 ab	0.08 ± 0.01 b

Values expressed as mean ± standard deviation. Different lowercase letters represent statistical differences ($p<0.05$).

Regarding dry biomass treatments T1 and T2 reached higher values than T3, which can be related to the lower nutrient concentration in 100% wastewater. This result contrasts with cell density data, where T2 and T3 showed better performance (Fig. 1a, 1b). Probably differences in cell volume related to the type of culture medium could explain these results, but this parameter was no determined in the present study The accumulated biomass data showed the same pattern of dry biomass, with T1 and T2 presenting values greater than T3 (Fig. 2). The same explanation given for dry biomass can be attributed to this result.

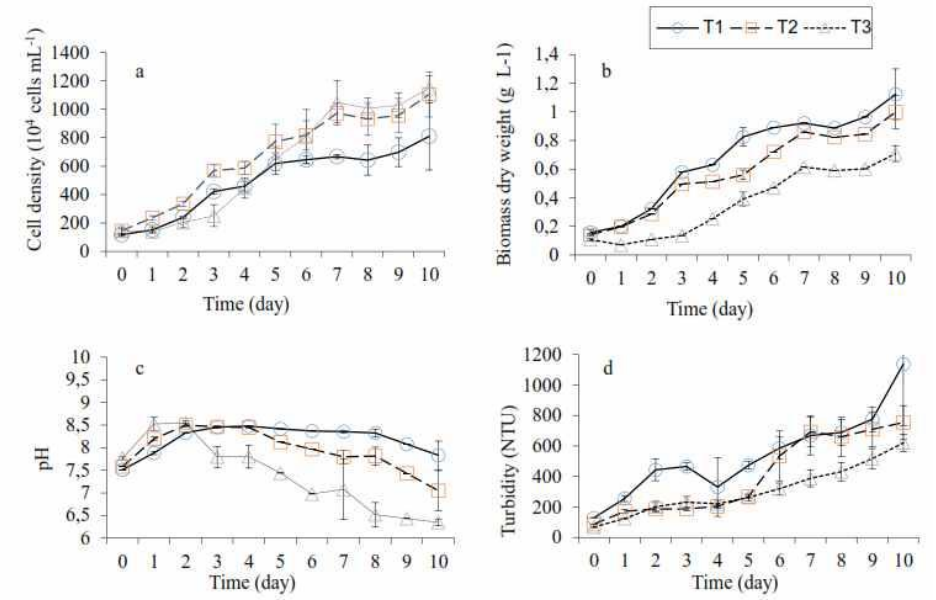

Figure 1. (a)Cell density variation (10^4 cells mL^{-1}) during cultivation period. (b)Dry biomass variation (g L^{-1}) during cultivation period. (c)pH variation during cultivation period. (d)Variation of turbidity in nephelometric units during the cultivation period. Each line refers to the average of four replicates of each experimental treatment. T1 - TAP Medium; T2 - 50% Wastewater; T3 - 100% Wastewater.

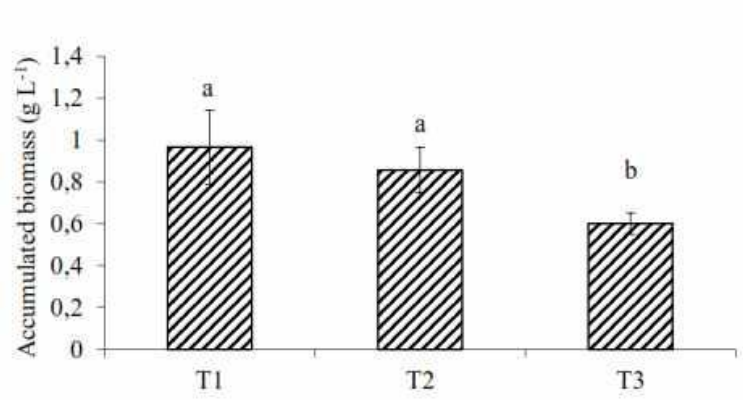

Figure 2. Accumulated biomass during cultivation period (g L^{-1}). Each bar refers to the average of four replicates of each treatment. T1 - TAP Medium; T2 - 50% Wastewater; T3 - 100% Wastewater. Different lowercase letters represent significant differences ($p<0.05$).

Regarding the productivity data, the cultivation in 100% wastewater (T3), presented lower results than using TAP medium. However, the addition of 50% wastewater to TAP medium showed no statistical difference with the other treatments, presenting an intermediate value (Table 2).

Jiang et al. (2011) cultivated *Nannochloropsis* sp. using conventional synthetic culture medium and an alternative culture medium composed of 50% wastewater, and reported a higher final dry biomass for the second one (0.16 and 0.21 g L^{-1}, respectively). These authors attributed this result to the presence of a higher concentration of organic carbon in the wastewater used. However, the same does not occur in the present research, suggesting that *C. acidophila* is not necessarily able to grow mixotrophically. In fact, recent work conducted by Souza et al. (2017) showed that this strain grew similarly in phototrophic and mixotrophic conditions and is not able to grow heterotrophically.

Evolution of pH showed a slight increase in the first two days followed by a decrease until the end of the experimental period for all the treatments, specially T3 (Fig. 1c). The three experimental treatments started with a pH value of 7.6 ± 0.1. At the end of the cultivation period, values of 7.8 ± 0.3, 7.0 ± 0.4 and 6.3 ± 0.1 were observed for the experimental treatments T1, T2 and T3, respectively. The mean pH value during the 10 days of cultivation period was 8.9 ± 0.1 for T1, 7.9 ± 0.1 for T2 and 7.4 ± 0.1 for T3. Considering the interest in use the wastewater at the same conditions in which it is discarded, this research tested the cultivation of acidophilic microalga without the acidification of the medium. However, studies should be conducted with the acidified alternative culture medium, which may increase the results presented here. The acidification of the culture medium can be done by introducing enriched air with high CO_2 concentrations (Babcock et al., 2002).

Turbidity increased with time reflecting the increase in cell density and dry biomass (Fig. 1d). Linear regression between dry biomass and turbidity showed good relationship, as can be seen in the equations bellow, where equations 1, 2 and 3 refer to treatments T1, T2 and T3, respectively. Dependent variable (y) is dry biomass (g L^{-1}) and independent variable is turbidity (NTU).

$$y=955.38x – 91.067 \ (r^2 = 0.8495) \quad (1)$$

$$y=781.93x – 73.058 \ (r^2 = 0.9419) \quad (2)$$

$$y=921.96x – 53.359 \ (r^2 = 0.9807) \quad (3)$$

Based on the high r^2 values, these equations can be used to estimate dry biomass from turbidity data.

Conclusions

The acidophilic microalga *C. acidophila* LAFIC-004 presented satisfactory growth performance when cultivated in treated domestic wastewater as an alternative culture medium, however, productivity was lower when compared to cultivation in conventional synthetic culture medium for *Chlamydomonas* spp. (TAP medium). Adding at least 50% of wastewater can be applied for best growth performance of this strain with additional environmental and economic benefits

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