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Chlorella minutissima cultivation with CO_2 and pentoses: Effects on kinetic and nutritional parameters

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ABSTRACT

CO_2 emissions and the large quantity of lignocellulosic waste generated by industrialized nations constitute problems that may affect human health as well as the global economy. The objective of this work was to evaluate the effects of using CO_2 and pentoses on the growth, protein profile, carbohydrate content and potential ethanol production by fermentation of *Chlorella minutissima* biomass. CO_2 and pentose supplementation can induce changes in the microalgal protein profile. A biomass production of 1.84 g.L^{-1} and a CO_2 biofixation rate of 274.63 mg.L^{-1}.d^{-1} were obtained with the use of 20% (v.v^{-1}) CO_2. For cultures with 20% (v.v^{-1}) CO_2 and reduced nitrogen, the carbohydrate content was 52.3% (w.w^{-1}), and theoretically, 33.9 mL.100 g^{-1} of ethanol can be produced. These results demonstrate that *C. minutissima* cultured with the combined use of CO_2 and pentoses generates a biomass with high bioenergetic potential.

1. Introduction

CO_2 emissions are among the most worrisome environmental problems, and the majority of these emissions result from burning oil-based fuels. CO_2 is considered the greatest contributor to global warming, and increased emissions have an effect on the increase in global temperature and rise in sea levels. The carbon capture and storage methods currently applied are not cost-effective and do not guarantee long-term sequestration. Therefore, the search for effective capture and storage methods is urgent. CO_2 biofixation by microalgae is an environmentally sustainable approach to capturing this gas, since the biomass produced in culture may be efficiently converted into several forms of bioenergy (Cheah et al., 2016; Ma et al., 2016; Razzak et al., 2013; Zeng et al., 2011).

Lignocellulosic waste is the most abundant source of renewable biomass, and when hydrolyzed, this type of waste generates significant amounts of xylose (Liguori and Faraco, 2016; Yang et al., 2014); therefore, the use of pentoses in microalgal culture has recently gained prominence. Some studies have been conducted since the first xylose uptake metabolic pathway in microalgae was reported for *Chlorella sorokiniana* cells by Zheng et al. (2014). Leite et al. (2015) were some of the first authors to report that a strain of the class Trebouxiophyceae (green algae) was capable of efficiently using D-xylose as a carbon source.

Small amounts of xylose are thought to potentiate the growth and production of bioproducts in microalgae of the genus *Chlorella*. Freitas et al. (2016) reported that the use of 20 mg.L^{-1} xylose increases the number of cells and the efficiency of photosystem II (PSII) in *C. minutissima*. Leite et al. (2016) reported rapid lipid accumulation, with the content doubling within 6–12 h under mixotrophic conditions that used D-xylose as carbon source in three *Chlorella* strains. Although there are studies on biological approaches to using pentoses and on CO_2 biofixation by photosynthetic microorganisms, the effects of the combined use of CO_2 and pentoses in microalgal cultures have not been reported.

Carbon, nitrogen, and phosphorus are the most important macronutrients for microalgal growth and represent the greater portion of the costs associated with culture media. The uptake of these compounds interferes with certain metabolic reactions and with the biosynthesis of various compounds, such as carbohydrates, proteins, and lipids (Costa and Morais, 2011; Sandoval et al., 2015). Therefore, the use of processes that use more than one residue as a source, such as CO_2 and pentoses, may yield sufficient amounts of carbon and, in addition to helping reduce CO_2 and industrial waste emissions, result in the production of biomass with several potential applications, including the production of biofuels such as bioethanol.

The objective of this work was to evaluate the effects of using CO_2 and pentoses on the growth parameters, protein profile, carbohydrate content and potential ethanol production of *C. minutissima*.

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2. Materials and methods

2.1. Microalgae and culture conditions

To perform the assays, *C. minutissima* from the Collection of the Laboratory of Biochemical Engineering of the Federal University of Rio Grande (FURG), Rio Grande do Sul, Brazil, was used. The microalgae were exposed to different CO_2 concentrations, a 50% reduction in the nitrogenous component and the addition of D-xylose and L-arabinose (Vetec Quimica, Sigma-Aldrich Corporation).

The strain was cultured in BMM medium (Watanabe, 1960) with the following composition: 0.250/0.125 $g.L^{-1}$ KNO_3 (giving 2.5 mM N and 1.25 mM N, respectively); 0.01 $g.L^{-1}$ $CaCl_2$; 0.075 $g.L^{-1}$ $MgSO_4{\cdot}7H_2O$; 0.075 $g.L^{-1}$ K_2HPO_4; 0.175 $g.L^{-1}$ KH_2PO_4; 0.025 $g.L^{-1}$ NaCl; 0.02 $g.L^{-1}$ $FeSO_4{\cdot}7H_2O$; and 1 mL of A_5 solution consisting of 2.86 $g.L^{-1}$ H_3BO_3, 1.81 $g.L^{-1}$ $MnCl_2{\cdot}4H_2O$, 0.222 $g.L^{-1}$ $ZnSO_4{\cdot}7H_2O$, 0.079 $g.L^{-1}$ $CuSO_4{\cdot}5H_2O$, and 0.015 $g.L^{-1}$ $NaMoO_4$. The assays were performed in 2.0 L vertical tubular photobioreactors with 1.8 L of working volume (Morais and Costa, 2007) and performed in duplicate, with triplicate of the analysis. These experiments were conducted in a temperature-controlled incubator at 30 °C, with an irradiance of 40 $\mu mol.m^{-2}.s^{-1}$ (provided by 40 W fluorescent lamps) and a light/dark photoperiod of 12 h, and the assays were carried out for 15 days.

For the evaluation of CO_2 biofixation, commercial gas was used in different proportions in a mixture with air at 10% and 20% ($v.v^{-1}$) with a specific flow rate of 0.05 vvm. The injections were performed every 18 min during the light period, with a duration of 59 s each. The conditions for the biofixation study are described in Table 1. Pentose (C5) addition was performed via a synthetic broth containing 19.16 $mg.L^{-1}$ D-xylose and 0.89 $mg.L^{-1}$ L-arabinose as previously described (Freitas et al., 2017).

2.2. Biomass concentration

Cell concentration was determined by spectrophotometry using a previously established standard curve for *C. minutissima*. This curve was obtained at 670 nm using a spectrophotometer (QUIMIS Q798DRM, Diadema, SP, Brazil) and correlated the relative optical density and dry biomass weight, as previously proposed by Costa et al. (2002).

2.3. Biomass volumetric productivity

The maximum biomass productivity (P_{max}, $g.L^{-1}.d^{-1}$) was determined according to Eq. (1), where X_t is the biomass concentration ($g.L^{-1}$) at time t (d), and X_0 is the biomass concentration ($g.L^{-1}$) at time t_0 (d).

$$P_{max} = \frac{(X_t - X_0)}{t - t_0} \tag{1}$$

2.4. Maximum specific growth rate

The maximum specific growth rate (μ_{max}, d^{-1}) was determined using an exponential regression applied to the logarithmic growth phase.

2.5. Determination the consumption of pentoses

The pentose consumption was determined using the methodology proposed by Somogyi (1952) and was applied to the supernatant obtained by centrifugation of the cultures at 27,000*g* for 10 min.

2.6. Biomass pentose conversion factor

The substratum conversion factor (xylose and arabinose) for the biomass ($Y_{X/S}$, $mg.mg^{-1}$) was calculated using Eq. (2), where X_0 and S_0 represent the biomass concentration and the substratum concentration, respectively, at the beginning of the culture, X_{max} is the maximum biomass concentration, and S_f is the final substratum concentration. The total pentose consumption (Sf = 0) was determined by item 2.5.

$$Y_{X/S} = \frac{(X_{max} - X_0)}{S_{max} - S_0} \tag{2}$$

2.7. Carbohydrate and protein content

For the quantification of carbohydrate and protein content, the biomass was separated from the culture medium and the washing water by centrifugation (Hitachi Himac CR-GIII, Tokyo – Japan) at 15,000*g* for 20 min.

The total carbohydrate concentration in the *C. minutissima* biomass was determined using the phenol–sulfuric method, with a standard glucose curve (DuBois et al., 1956).

The total protein concentration in the biomass was determined at the end of the assays using the colorimetric method proposed by Lowry et al. (1951), after the thermal and alkaline pre-treatment (heating with addition of NaOH at 100 °C for 5 min) of the *C. minutissima* biomass.

The results of the characterization of the biomass obtained from cultures with pentoses (CC5) relative to that obtained from control cultures (CC) were compared according to the relationship proposed by Deamici et al. (2016). The relationship is described by Eq. (3), where R (R_C or R_P) corresponds to the percentage difference in the results obtained with pentoses relative to the results obtained in the control cultures.

$$R = \frac{CC5\text{-}CC}{CC} \cdot 100 \tag{3}$$

Table 1
Results for the maximum cell concentration (X_{max}, $g.L^{-1}$), maximum productivity (P_{max}, $g.L^{-1}.d^{-1}$), maximum specific growth rate (μ_{max}, d^{-1}), biomass pentose conversion factor ($Y_{X/S}$, $mg.mg^{-1}$), maximum CO_2 biofixation rate (R_{CO2max}, $mg.L^{-1}.d^{-1}$) and maximum CO_2 biofixation efficiency (E_{CO2max}, $\%w.w^{-1}$) (mean ± standard deviation – *n* = *3*).

Assays	Culture Conditions	X_{max} ($g.L^{-1}$)	P_{max} ($g.L^{-1}.d^{-1}$)	μ_{max} (d^{-1})	$Y_{X/S}$ ($mg.mg^{-1}$)	R_{CO2max} ($mg.L^{-1}.d^{-1}$)	E_{CO2max} ($\%w.w^{-1}$)
1	10% CO_2/2.5 mM N	1.59 ± 0.05^b	0.15 ± 0.02^a	0.32 ± 0.04^b	–	250.39 ± 34.47^a	70.7 ± 9.70^a
2	10% CO_2/1.25 mM N	1.07 ± 0.04^a	0.14 ± 0.01^a	0.52 ± 0.02^a	–	242.14 ± 5.25^a	68.4 ± 1.60^a
3	10% CO_2/1.25 mM N/19.16 $mg.L^{-1}$ Xylose and 0.89 $mg.L^{-1}$ Arabinose	1.08 ± 0.03^a	0.14 ± 0.01^a	0.53 ± 0.02^a	$0.04 \pm < 0.01^a$	247.98 ± 1.24^a	70.1 ± 0.35^a
4	20% CO_2/2.5 mM N	1.84 ± 0.07^b	0.16 ± 0.01^a	$0.41 \pm 0.03^{a,b}$	–	274.63 ± 31.77^a	38.8 ± 4.49^b
5	20% CO_2/1.25 mM N	1.09 ± 0.01^a	0.14 ± 0.01^a	0.52 ± 0.03^a	–	247.67 ± 16.78^a	35.0 ± 2.37^b
6	20% CO_2/1.25 mM N/19.16 $mg.L^{-1}$ Xylose and 0.89 $mg.L^{-1}$ Arabinose	1.14 ± 0.02^a	0.15 ± 0.01^a	0.74 ± 0.01^c	$0.04 \pm < 0.01^a$	266.26 ± 13.54^a	37.6 ± 1.91^b

The presence of the same superscript letter in the same column indicates that the means are not significantly different at a 95% confidence level ($p > 0.05$).