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PRODUCTION OF LIPIDS AND CARBOHYDRATES FROM THE CULTIVATION OF *Chlorolobion braunii* IN PRODUCED WATER

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ABSTRACT

Produced water (PW) is a complex waste byproduct considered an alternative for microalgae cultivation, aiming to reduce the toxic potential of this effluent while also cutting the costs of nutrients and water used in microalgae media, and promoting the production of biomass with an industrially valuable composition. This study aims to produce *C. braunii* biomass with high lipid and carbohydrate content by cultivating the freshwater chlorophyte microalga ***Chlorolobion braunii*** in produced water. A medium of 50% BG-11 and 50% untreated produced water was used, with cultures maintained for 21 days. Maximum production was 0.274 g L^{-1} in the control and 0.156 g L^{-1} in PW50%. The production of total lipids and carbohydrates was similar in control cultures (13.36% and 37.08%) and PW50% (16.44% and 24.51%). The main fatty acids in PW50% were palmitic acid (34.59%), γ-linolenic acid (17.62%), and α-linolenic acid (17.02%). Therefore, using PW as a medium for cultivating ***C. braunii*** is a viable and promising alternative, reducing water and nutrient costs and improving biomass composition, making it more appealing for the biofuel industry.

Keywords: Chlorophyte. Freshwater. Bioremediation. Biofuel. Composition.

1 INTRODUCTION

Produced water (PW) is an effluent from the petroleum industry, obtained during the oil recovery process. It is estimated that the ratio between the extraction of produced water and oil is 3:1 (Al-Ghouti et al., 2019). In 2023, Brazil alone produced an average of 3.402 million barrels of oil per day, according to the National Agency of Petroleum, Natural Gas, and Biofuels (ANP). PW is considered highly polluting due to the presence of organic compounds such as aliphatic and aromatic hydrocarbons and heavy metals, phenols, and radionuclides (Amakiri et al., 2022). Due to its composition, processes are necessary to reduce the concentrations of contaminants before disposal. However, PW contains other components, such as nitrate and phosphate, which can be used as sources of nitrogen and phosphorus for the growth of microorganisms, such as microalgae (Silva et al., 2023). The cultivation of microalgae in wastewater, such as produced water, has been considered a potential alternative for treating these effluents, as well as for reducing the costs of nutrients and water during cultivation, synthesizing biomolecules of industrial interest, and allowing the production of biodiesel with properties similar to or better than biofuels already established in the market (Borges et al., 2024). Thus, this study aimed to produce biomass of the freshwater chlorophyte microalga *Chlorolobion braunii* with high lipid and carbohydrate content.

2 MATERIAL & METHODS

Obtaining Produced Water (PW), Cultivation Conditions, and Growth Parameters:

Produced water (PW) was supplied by an oil exploration company located in Mata de São João (Bahia) and did not undergo any pre-treatment process before use. The strain of the microalga ***C. braunii*** (Nägeli) Komárek 1979 was obtained from the Freshwater Microalgae Culture Collection of the Federal University of São Carlos and is registered as CCMA-UFSCar 455. Cultivation was carried out using a concentration of 50% produced water + 50% BG11 (PW50%) in an Erlenmeyer-type photobioreactor with a working volume of 880 mL in a BOD-type climatic chamber with a 12-hour light/dark photoperiod, illumination of 124.87 µmol photons m^{-2} s^{-1}, and a temperature of 25°C for 21 days. Aeration of the cultures was provided by an air pump with a flow rate of 50 L h^{-1}, and the cultures were performed in triplicate. At the end of the cultivations, they were centrifuged at 10,000 rpm for 10 minutes at 4°C to separate the biomass and the residual medium. The biomasses were frozen, lyophilized, and used for biochemical characterization analyses. Biomass production (X) was determined daily through optical density (OD) by spectrophotometry at a wavelength (λ) of 680 nm, following the standard curve of the relationship between dry mass and absorbance (Costa et al., 2022).

Biomolecules and Fatty Acid Composition:

The determination of carbohydrates present in the biomass followed the methodology proposed by Dubois et al. (1956). The total lipid content of the biomass was determined according to Folch et al. (1957). Fatty acids were transmethylated with hexane boron trifluoride, and the individual fatty acids of the biomass were identified by gas chromatography (Clarus 680; Perkin Elmer®) equipped with a flame ionization detector (Nascimento et al., 2014).

Statistical Analysis

The results were analyzed using STATISTICA 10 software and subjected to the Shapiro-Wilk normality test. The data were then subjected to analysis of variance (ANOVA), followed by Tukey's test, considering a 95% confidence level for comparing the means. The results are shown as mean ± standard deviation.

3 RESULTS & DISCUSSION

The microalgae went through an initial adaptation phase in both conditions, lasting for 2 days, before entering an exponential growth phase from the 3rd day onwards (0.182 and 0.061 g L^{-1}, respectively) (Figure 1a). Compared to the control, PW50% exhibited a shorter period of exponential growth, extending only until the 15th day, followed by a stationary phase from the 16th to the 19th day, and ultimately, a phase of cell death from the 20th day onward. Maximum biomass production occurred on the 18th day (control, 0.274 g L^{-1}) and the 15th day (PW50%, 0.156 g L^{-1}). Interestingly, there was no statistical difference between the biomass production values, suggesting that PW50% is a feasible alternative for cultivating *C. braunii*.

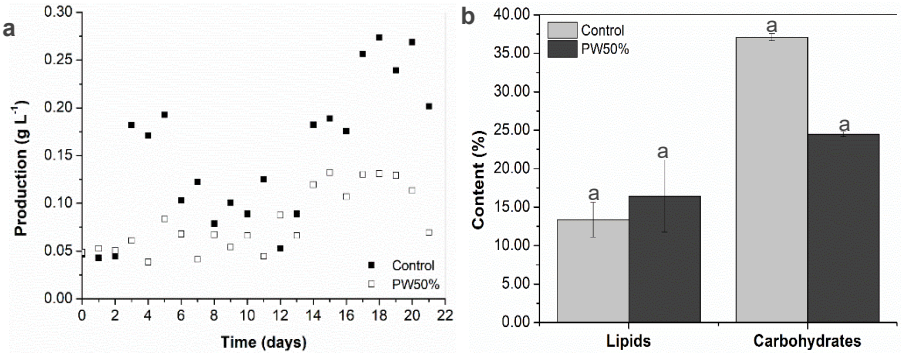

Figure 1 Biomass production (a) and composition (b) of *C. braunii* in medium containing 50% BG-11 and 50% produced water (PW).

There was no significant difference ($p < 0.05$) between the total lipid contents of the control (13.36%) and PW50% (16.44%), as well as the carbohydrates in the control (37.08%) and PW50% (24.51%) in the *C. braunii* biomass. These results indicate that cultivation in PW does not have negative impacts on the microalgae's metabolism, providing the necessary nutrients for growth and biomolecule production. The lipid content obtained in this study was higher than that obtained by Silva et al. (2022) for the chlorophyte *Chlorella vulgaris* (9.92% in PW30%). Silva et al. (2023) obtained C. vulgaris biomass with 40.19% carbohydrates and 21.53% lipids after cultivation with PW.

The predominantly found fatty acids were palmitic acid (C16:0), linoleic acid (C18:2ω6t), and γ-linolenic acid (C18:3ω6) (Table 1). According to Hopkins et al. (2019), different species of microalgae, including those of the chlorophyte class, show an increase in the production of fatty acids C16:0, C18:2, and C18:3 when cultivated in PW. This is attributed to the influence of the salinity of this waste on the metabolic and osmotic regulation of the cells present in the medium. Moreover, microalgae produce these fatty acids in larger quantities under stress conditions (Williams; Laurens, 2010) as they are common constituents of cell membranes, and their synthesis serves as a protective mechanism to maintain the integrity of these cellular structures. The presence of long-chain fatty acids such as C16:0 confers favorable properties to the produced biodiesel, as it reduces ignition time and smoke generation (Ramos et al., 2009).

Table 1 Fatty acids profile of *C. braunii* cultivated in BG-11 medium (control) and PW50%.

Class	Fatty acid	%	
		Control	PW50%

Saturated	C14:0	2.09 ± 0.32	1.71 ± 0.97
	C16:0	46.56 ± 1.41	34.59 ± 1.68
	C18:0	1.66 ± 1.68	3.73 ± 0.99
	Sum (%)	**50.31 ± 0.59[a]**	**40.02 ± 0.28[b]**
Monounsaturated	C14:1ω5	1.36 ± 0.04	ND
	C15:1	1.08 ± 0.11	ND
	C16:1ω7	2.19 ± 0.49	1.55 ± 0.64
	C17:1ω5	1.69 ± 0.11	4.39 ± 0.98
	C18:1ω9t	2.45 ± 0.64	4.50 ± 1.33
	Sum (%)	**8.77 ± 0.87[a]**	**10.44 ± 0.29[a]**
Polyunsaturated	C18:2ω6c	1.07 ± 0.24	ND
	C18:2ω6t	21.04 ± 0.74	14.92 ± 1.99
	C18:3ω6	12.06 ± 0.05	17.62 ± 0.37
	C18:3ω3	6.78 ± 0.75	17.02 ± 1.05
	Sum (%)	**40.94 ± 0.28[a]**	**49.55 ± 0.57[b]**
Total (%)		**100.00**	**100.00**

± indicates the standard deviation. Equal letters in the same line indicate that there was no significant difference between the experiments at 95% confidence (p>0.05).

4 CONCLUSION

Produced water (PW) did not exert any toxic effects on the cellular metabolism of *C. braunii*, as evidenced by the uninterrupted exponential cell growth observed over 15 days of cultivation. Additionally, the production of biomolecules such as lipids and carbohydrates remained unaffected, reaching values similar to those observed in standard synthetic media. The stimulation of polyunsaturated fatty acids (C18:2 and C18:3) composition during cultivation in PW is particularly noteworthy, enhancing both cellular metabolism and the production of high-quality biodiesel. Thus, the use of PW as a cultivation medium for the chlorophyte *C. braunii* proves to be a viable and promising alternative, suitable for integration into integrated biorefinery systems, facilitating a high degree of effluent conversion (50% PW) into substrates for obtaining carbohydrates and lipids that can be bioconverted into biofuels.

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