



Optimized Cultivation of *Chlorolobion braunii* in Desalination Waste Streams for Bioremediation and Biodiesel Production

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Abstract

The valorization of saline concentrate and brackish water is a key strategy for the sustainable management of water resources, particularly in semi-arid regions. Cultivating microalgae in these effluents mitigates environmental impacts and enables high-value biomass production, offering potential for the generation of bioproducts and added benefits for regional agricultural systems. This study aimed to optimize biomass production and characterize the microalga *Chlorolobion braunii* using a combination of brackish water and saline concentrate. It used urea as the nitrogen source, without pre-treatment or the addition of synthetic medium, which are strategies not previously reported. Additionally, the potential of this biomass for biodiesel production was evaluated. The experiments were conducted in Erlenmeyer-type bioreactors (1 L), and the best biomass production condition was determined using a Central Composite Design (CCD) with the following factors: volume of saline concentrate in brackish water (0–100%) and urea concentration (0.00–0.08 g·L⁻¹). The best treatment was the experiment with 50% brackish water and 50% saline concentrate, yielding 0.556 g·L⁻¹ of biomass. This biomass had high contents of carbohydrates (21.14%) and lipids (29.40%), while achieving bioremediation of 86.25% nitrates (NO₃⁻), 79.17% phosphate (PO₄³⁻), and a 23.18% reduction in salinity. The biomass exhibited a high content of fatty acids, with 24.94% C16:0 (palmitic) and 38.44% C18:1n9c (oleic), producing biodiesel that meets American (ASTM D6751) and Brazilian (ANP) standards, particularly with respect to quality parameters such as cetane number, iodine value, and cold filter plugging point. This study optimized the biomass production of the microalga *Chlorolobion braunii*. It demonstrated the feasibility of using a combination of brackish water and saline concentrate without pre-treatment or synthetic medium, resulting in high biomass productivity and elevated biomolecule levels that can serve as substrates for high-value bioproduct generation. This approach provides a dual solution for waste management and renewable energy production.

Keywords Saline stress · Urea · Microalgae · Nutrient · Response surface methodology

Introduction

Global climate change, caused by the excessive emission of greenhouse gases, has altered the atmospheric chemical composition, directly impacting the climate. These changes result in shifts in precipitation patterns and the availability of water resources [1]. In Brazil, the Northeast region is widely recognized for having the lowest water availability, often attributed to the poor quality of its available water resources [2]. According to the National Water and Basic Sanitation Agency [3], from October to December 2024, the drought in the Northeast intensified by two severity categories. The *sertão Nordestino*, a semi-arid region facing a critical water scarcity situation, relies on brackish groundwater

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desalination as a solution to obtain potable water in various localities [4]. According to the study, this process is carried out through membrane systems that treat raw water via reverse osmosis, whose main advantage is the high quality of the treated water.

Reverse osmosis technology is not only used in the Brazilian semi-arid region but is also employed in various water-scarce regions around the world, such as the Middle East, including coastal and arid areas of Iran [5], as well as other regions that utilize this technology, such as Spain [6], North Africa [7]. It is also used in the southwestern United States (e.g., Florida [8]), and in parts of Australia, such as the state of Victoria [9], where desalination serves as an alternative to ensure a supply of potable water. Despite its efficiency, the technology poses environmental challenges regarding the management of the saline waste generated in the process.

According to the Ministry of Integration and Regional Development [10], 3.6 million liters of potable water are produced daily, benefiting 218,000 people in the rural areas of the Brazilian semi-arid region. However, 40 to 60% of the total brackish water extracted from the well is rejected as part of the process, forming what is known as saline concentrate [11, 12]. This byproduct is rich in inorganic minerals such as Cl^- , Na^+ , and Ca^{2+} , as well as other nutrients like nitrogen (N) and phosphorus (P), which are essential for microalgal growth [13, 14].

The saline concentrate produced by the plants installed in the Northeast region can potentially provide water and nutrients for microalgal growth due to its chemical composition, with carbon, nitrogen, and phosphorus being the main macronutrients in microalgal metabolic processes [12]. Carbon is essential for the formation of organic substances in cells (proteins, carbohydrates, nucleic acids, vitamins, lipids, etc.), while phosphorus is associated with energy transfers and the formation of structural molecules in cells, such as ATP, phosphorylated sugars, nucleic acids, and phosphoenzymes [15, 16]. Nitrogen is an essential element in cellular structural substances, including proteins, nucleic acids, and photosynthetic pigments, such as chlorophylls and phycobilins. The primary forms of nitrogen used in culture media are nitrate salts (NaNO_3 , KNO_3), ammonium salts (NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$), and urea [16].

The use of saline concentrate and urea as nutrient sources can reduce operational costs by partially or completely replacing synthetic culture media and conventional nitrogen salts. Using effluents as cultivation media lowers expenses associated with freshwater supply and nutrient supplementation [17], contributing to favorable economic performance and demonstrating the scalability potential of microalgae cultivation systems integrated with desalination processes. Urea ($\text{CO}(\text{NH}_2)_2$) is one of the nitrogen sources

with the highest nitrogen content, containing approximately 46% nitrogen by weight [18]. This high concentration, combined with its lower cost (US\$ 0.39 per kg) [19] compared to sodium nitrate (US\$ 1.31 per kg) [20], the most commonly used source in cultivation media, makes urea a promising and economically accessible alternative. Moreover, its use as a nitrogen source has been associated with increased biomass production [21].

Aerobic mixotrophic cultivation enables microalgal cells to exhibit greater metabolic flexibility, generating energy through both photosynthesis (utilizing light) and aerobic respiration (utilizing oxygen and organic compounds), thereby enhancing biomass productivity [22]. Furthermore, according to the study, mixotrophic cultivation can utilize alternative and low-cost carbon sources, such as wastewater, reducing overall costs and promoting a more sustainable approach.

In addition, microalgae utilize nutrients present in wastewater, such as saline concentrate, performing bioremediation while simultaneously sequestering carbon dioxide (CO_2) [23, 24]. The biomass produced in this process can be used to produce biomolecules and high-value bioproducts, such as biofuels [25, 26]. *Chlorolobion braunii*, a freshwater microalga belonging to the class *Trebouxiophyceae* [27], which includes green, photosynthetic organisms [28], has potential for biotechnological applications, including biofuel production, such as biodiesel, due to its high lipid content [29]. Additionally, *C. braunii* can be employed in wastewater treatment systems, contributing to the removal of nutrients and pollutants from residual waters. However, it remains an understudied species, with limited research on its biology, metabolism, and potential biotechnological applications. Its growth capacity, biochemical composition, and ability to produce bioproducts suggest that future investigations may uncover new opportunities for its utilization. In this context, despite the potential of saline effluents as alternative cultivation sources, there are no studies that have optimized the cultivation of *C. braunii* in mixed brackish water and saline concentrate media without using synthetic nutrients.

The present study pioneers the use of *Chlorolobion braunii* with saline concentrate and brackish water, aiming to optimize the biomass production using a mixed culture medium of brackish water and saline concentrate and urea as the nitrogen source. In addition, the study characterizes the produced biomass and evaluates its suitability for biodiesel production by assessing lipid yield and fuel quality. By employing a non-synthetic cultivation medium (brackish water + saline concentrate), this work aims to reduce production costs while providing unprecedented data on the productivity, lipid composition, and biodiesel potential of a rarely studied microalgal species, contributing to

sustainable strategies for desalination waste valorization and renewable energy.

Materials and Methods

Collection and Characterization of Wastewaters

The saline concentrate (SC) and brackish water (BW) used as alternative culture media were collected in the Mandassaia II region, a settlement belonging to Riachão do Jacuípe (11°48' 42"S, 39°23' 9"W), Bahia, Brazil. The saline concentrate and brackish water were used in the experiments immediately after collection to avoid variations in their physicochemical characteristics without any prior treatment. The saline concentrate and brackish water were analyzed for nitrate (NO_3^-) composition (SM 4500B), bicarbonate alkalinity (CaCO_3) (SM 2320 A/B), phosphate (PO_4^{3-}) (SM 411B), and total salinity (SM 2520 C). All the analyses described are established by APHA (2012) [30]. Removal efficiency was obtained using Eq. (1).

$$\text{Removal Efficiency (\%)} = \frac{C_b - C_a}{C_b} * 100\% \quad (1)$$

Where C_b is the concentration of the nutrient before cultivation and C_a is the concentration after cultivation.

Microalgae, Inoculum, and Experimental Conditions

The microalgal strain used in the study was *Chlorolobion braunii* 455, obtained from the microalgal culture collection of the Department of Botany at the Federal University of São Carlos (UFSCar), São Paulo, Brazil. Previous studies have shown that *Chlorolobion braunii* exhibits rapid growth and metabolic adaptability when cultivated under alternative conditions, including industrial effluents [31–33]. These studies also reported the ability of this strain to accumulate high-value biomolecules, such as lipids and other metabolites. However, despite this demonstrated potential, no study has optimized the cultivation of *C. braunii* in mixed media comprising brackish water and saline concentrate without the addition of synthetic nutrients. Therefore, this study aims to fill this gap by evaluating and optimizing growth performance, lipid accumulation, and biodiesel quality of *C. braunii* cultivated in desalination effluents.

The inoculum was cultivated in BG-11 medium with the following composition: 1500 mg L^{-1} of sodium nitrate (NaNO_3); 40 mg L^{-1} of potassium phosphate dibasic trihydrate ($\text{K}_2\text{HPO}_4 \cdot 3 \text{H}_2\text{O}$); 75 mg L^{-1} magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$); 20 mg L^{-1} of sodium carbonate (Na_2CO_3); 27 mg L^{-1} of calcium chloride (CaCl_2); 6 mg L^{-1}

citric acid monohydrate; 6 mg L^{-1} ammonium ferric citrate; 1 mg L^{-1} Na_2EDTA ; and 1 mL of trace metal solution (containing 2.86 mg L^{-1} H_3BO_3 ; 1.81 mg L^{-1} $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$; 0.222 mg L^{-1} $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$; 0.079 mg L^{-1} $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$; 0.050 mg L^{-1} $\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$; 0.39 mg L^{-1} $\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$). In the exponential growth phase of the culture, the biomass was concentrated by centrifugation ($11200 \times g$ and 4°C for 5 min), washed with distilled water, and centrifuged again under the same conditions to remove any salts present in the biomass. The temperature was maintained at $25 \pm 2^\circ\text{C}$, and the evaporated water from the cultures was replenished daily before sample removal. The light-dark cycle was set to 12:12 h, with a light intensity of $124.87 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ provided by LED tubes and constant aeration at 45 L h^{-1} . The air was pre-filtered with glass wool to remove contaminants.

The biomass production experiments were carried out in a BOD incubator (SL200/334) using Erlenmeyer-type photobioreactors with a volume of 1.0 L and a working volume of 0.8 L. The mixotrophic cultures had an initial biomass concentration of 0.2 g L^{-1} , and the experiment lasted 20 days. The cultivation conditions were the same as those of the inoculum, except for the culture medium. At the end of the cultures, the biomass samples were centrifuged (Hitachi Himac CR-GIII, Tokyo, Japan) at $11,200 \times g$ and 4°C for 5 min, then resuspended with distilled water to remove salts and impurities. The biomass was then frozen (-80°C), lyophilized for 48 h, and stored at -20°C , together with the supernatant, until characterization [34].

Experimental Design

Response surface methodology was applied to evaluate the condition producing the highest biomass concentration using brackish water, saline concentrate, and urea. This set of techniques aims to determine optimal points (maximum biomass concentration). The optimization of the maximum biomass concentration in the experiments was analyzed based on the experimental design, specifically the Central Composite Design (CCD), evaluating the variation of two independent factors: nitrogen concentration (using urea as an alternative source) and the percentage of saline concentrate in brackish water. Urea was selected instead of conventional nitrogen sources, such as sodium nitrate, due to its lower cost, wide availability, and high solubility, which enhance the economic feasibility of large-scale cultivation [35].

Using Statistica 13.3[®] software (TIBCO Inc., USA), the CCD was carried out with 12 experiments, with two levels (2^2), four axial points, and the repetition of the central point, utilizing Erlenmeyer-type photobioreactors with a volume of 1.0 L and a working volume of 0.8 L. Table 1 shows the coded and decoded values analyzed for biomass production optimization.

Table 1 Coded level of the CCD

Factors	Unit	Coded level and value				
		-1.41421	-1	0	1	1.41421
Saline concentrate (SC)	%	0.00	14.645	50.00	85.355	100.00
Urea concentration (Ur)	g L ⁻¹	0.00	0.011	0.04	0.068	0.08

The quadratic model for predicting biomass production is described by Eq. (2).

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \beta_{ij} X_i X_j + \dots \quad (2)$$

Where Y is the estimated response, X are the factors, β_0 is the constant coefficient, and β are the coefficients associated with the model's factors.

The analysis of variance (ANOVA) with Fisher's F test at a 95% confidence level was used to determine the significance of the regression model of the data. The effects of the independent variables were evaluated, including the surface plot of the statistically significant variables and the contour plot. The suitability of the regression model was evaluated using the coefficient of determination (R^2 and R^2_{adj}), while the significance was assessed through the p-value and F-value obtained from the analysis of variance.

Growth Parameters and pH

The biomass concentration (X) of *C. braunii* was determined every 24 h by measuring optical density (OD) using a digital spectrometer (PerkinElmer, Lambda 35 UV/VIS) with a wavelength (λ) of 680 nm. A specific standard curve for *C. braunii* was used to correlate concentration with optical density ($0.2679 \times OD_{680} + 0.0137$) according to [36].

The growth parameters were determined based on the biomass concentration values. The following parameters were calculated: maximum biomass concentration (X_{max} ; g L⁻¹), maximum biomass productivity (P_{max} ; g L⁻¹ d⁻¹), final productivity (P_{final} ; g L⁻¹ d⁻¹), and specific growth rate (μ_{max} ; d⁻¹). Productivity was determined according to Eq. (3), where X_t is the biomass concentration in g L⁻¹ at time t in days and X_0 is the initial concentration. μ_{max} (d⁻¹) was determined as the rate of change in the linear regression of the logarithm of growth during the exponential phase over time (lnX vs. t). pH was monitored daily using a digital pH meter (Tecnal Tec-5).

$$P_X = \frac{X_t - X_0}{t - t_0} \quad (3)$$

Biomass Biochemical Composition

The lyophilized biomass after cultivation was characterized for its total carbohydrate content, determined according to

the methodology proposed by [37], using phenol and sulfuric acid to hydrolyze the higher-molecular-weight carbohydrates and break the cell wall, extracting sugars. The content was determined using a glucose standard curve. The total lipid percentage was determined by extraction at room temperature (25 ± 2 °C) of apolar lipids with chloroform: methanol (2:1) and polar lipids with methanol: water (2:1), according to the method suggested by [38]. The protein percentage was determined from the total nitrogen content using the Kjeldahl method [39], where sulfuric acid converts the sample's nitrogen into ammonium ions. A conversion factor of 4.72 [40] for Chlorophyceae was used to determine the protein percentage.

Fatty acid Methyl Esters (FAME) Composition and Biodiesel Production

Fatty acid methyl esters (FAME) were quantified by gas chromatography (DB-FFAP 30 m $\times$ 0.25 mm $\times$ 0.25 μ m) with a flame ionization detector (GC-FID Clarus 680; Perkin-Elmer). A 1 μ L sample aliquot was injected in split mode (1:50) in triplicate. The temperature programming for the injector and detector was set at 250 °C and 280 °C, respectively. The temperature program began at 150 °C for 16 min, then increased at a rate of 2 °C min⁻¹ to 180 °C, held for 25 min. Subsequently, the temperature was further raised to 235 °C at 5 °C min⁻¹ and maintained for 25 min. Helium was used as the carrier gas at 1.0 mL min⁻¹. FAME identification was performed by comparing the retention times of the sample with those of the standard chromatogram (C4-C24, 189-19-AMP, Sigma-Aldrich). The peak areas were determined following the method proposed by [41], using Clarus Chromatography software for normalization. The results are expressed as a percentage (%).

Evaluation of Biodiesel Properties

The parameters determining biodiesel quality were assessed based on the molecular structures, size, quantity, and/or position of double bonds in the fatty acids composing the lipids. The cetane number (CN), saponification value (SV), iodine value (IV), degree of unsaturation (DU), cold filter plugging point (CFPP), and long-chain saturated factor (LCSF) were calculated as per [42]. The results were compared with the limits established by the current standards in Brazil, the United States, and Europe. The cetane number,

saponification value, and iodine index were calculated according to Eqs. (4), (5), and (6).

$$CN = 46.3 + \frac{5458}{VS} - 0.225 \times IV \quad (4)$$

$$SV = \sum \frac{560 \times N}{M} \quad (5)$$

$$IV = \sum \frac{254 \times D \times N}{M} \quad (6)$$

Where D is the number of unsaturations, M is the molecular mass, and N is the percentage of each FAME. The degree of unsaturation, cold filter plugging point, and long-chain saturated factor were calculated according to Eqs. (7), (8), and (9).

$$DU = MUFA + 2 \times PUFA \quad (7)$$

$$CFPP = 3.1417 \times LCSF - 16.477 \quad (8)$$

$$LCSF = (0.1 \times C16 : 0) + (0.5 \times C18 : 0) + (1 \times C20 : 0) + (1.5 \times C22 : 0) + (2 \times C24 : 0) \quad (9)$$

Where MUFAs are the monounsaturated fatty acids, PUFAs are the polyunsaturated fatty acids, C16:0, C18:0, C20:0, C22:0, and C24:0 are the weight percentages of palmitic, stearic, arachidic, behenic, and lignoceric acids, respectively.

Statistical Analysis

The assays were performed in triplicate, and the results are presented as means $\pm$ standard deviation. All data were statistically analyzed, first evaluating normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test > 0.05). Then, a one-way Analysis of Variance (ANOVA) was conducted with a 95% confidence level. Significant differences were tested utilizing the Tukey post hoc test, using Statistica 13.3[®] software (TIBCO Inc., USA).

Results and Discussion

Biomass Production Optimization

Table 2 presents the experiments combining the percentage of saline concentrate and urea concentration to optimize the maximum biomass production. It can be observed that the highest biomass production (X_{max}) corresponds to experiment 7, which has 50% SC and 50% BW. These conditions are directly associated with the effects of high salinity

Table 2 Experimental design with the real and coded values of the saline concentrate percentage, brackish water, and urea concentration, and the results of the maximum production

Experiment	SC (%)	Ur (g L ⁻¹)	BW (%)*	X _{max} (g L ⁻¹)
1	14.645 (-1)	0.011 (-1)	85.355	0.465
2	85.355 (1)	0.011 (-1)	14.645	0.176
3	14.645 (-1)	0.068 (1)	85.355	0.238
4	85.355 (1)	0.068 (1)	14.645	0.255
5	0.0 (-1.41)	0.040 (0)	100.0	0.464
6	100.0 (1.41)	0.040 (0)	0.0	0.190
7	50.0 (0)	0.0 (-1.41)	50.0	0.556
8	50.0 (0)	0.080 (1.41)	50.0	0.223
9	50.0 (0)	0.040 (0)	50.0	0.523
10	50.0 (0)	0.040 (0)	50.0	0.485
11	50.0 (0)	0.040 (0)	50.0	0.501
12	50.0 (0)	0.040 (0)	50.0	0.381

* Percentage of brackish water used to complete the cultivation volume; SC saline concentrate, BW brackish water, Ur urea

(4.96 g L⁻¹) and the absence of urea, which influence the growth and biomass of *C. braunii*.

The linear, quadratic, and interaction effects of the regression are presented in Table 3. It can be observed that the quadratic effect of the saline concentrate percentage was significant (p-value < 0.05), indicating the presence of a critical region, which is a maximum region (0.0–50% saline concentrate; 0.0–0.04 g L⁻¹ urea). The linear effects are significant (p-value < 0.05) and indicate a negative effect on maximum biomass production. The transition from the minimum level of saline concentrate percentage to the maximum level causes the biomass concentration to decrease by 0.1648 g L⁻¹. The reduction in biomass concentration observed is associated with the increase in salinity, provided by the increase in the concentrate percentage in the medium (0 to 100%). Under salt stress, available energy is redirected to processes, such as osmoregulation at the expense of cell growth and photosynthesis, leading to reduced growth and biomass yield, as observed by [43].

According to [44], a saline medium reduces the osmotic potential, hindering water absorption and causing cell dehydration. Moreover, it causes ionic stress due to an imbalance in ionic homeostasis, mainly from the competition between Na⁺ and K⁺ ions for absorption channels. This results in K⁺

Table 3 Effects on maximum biomass production

Factor	Effect	t value	p value
Constant	0.472	14.980	6.45×10^{-4}
Saline concentrate linear (X_{SC})	-0.164	-3.690	0.034
Urea concentration linear (X_{Ur})	-0.154	-3.460	0.035
Saline concentrate quadratic (X_{SC}^2)	-0.182	-3.660	0.040
Urea concentration quadratic (X_{Ur}^2)	-0.120	-2.410	0.095
Interaction (X_{SC})* (X_{Ur})	0.152	-2.420	0.093

X_{SC} percentage of saline concentrate, X_{Ur} urea concentration

deficiency in the cytoplasm, compromising enzymatic processes and membrane potential. The toxic accumulation of Na^+ ions can damage organelles and proteins by disrupting ionic homeostasis in chloroplasts [45]. When cultivating *Desmodesmus* sp. KNUA024, a green microalga, in saline concentrate with the addition of 4.5 mg L^{-1} phosphorus [46], observed a similar reduction in biomass production compared to the control (BG-11), from 1.0 g L^{-1} to 0.6 g L^{-1} of dry biomass.

Similarly, the transition from the minimum level of urea concentration in the medium to the maximum level causes a reduction of 0.1545 g L^{-1} in the biomass concentration. Although urea stimulates the growth of microalgal biomass and participates in the synthesis of biomolecules [21, 47], adding it without proper phosphorus control in the medium can result in the accumulation of reactive oxygen species (ROS), altering processes such as ATP and phospholipid synthesis, leading to reduced cell division [48]. The interaction effect of the factors and the quadratic effect of urea concentration did not show statistical significance ($p\text{-value} > 0.05$).

The second-order model obtained for maximum biomass production (X_{max}) is presented according to Eq. (10), whereby statistically non-significant parameters were retained.

$$X_{\text{max}} = 0.473 - 0.082X_{\text{SC}} - 0.091X_{\text{SC}}^2 - 0.077X_{\text{Ur}} - 0.060X_{\text{Ur}}^2 - 0.076X_{\text{SC}}X_{\text{Ur}} \quad (10)$$

Table 4 shows the analysis of variance for maximum biomass production, in which the coefficient of determination (R^2) and adjusted coefficient of determination (R^2_{adj}) are equal to 88.48% and 69.67%, respectively. This suggests that the model has high explanatory power, as high values of both R^2 and adjusted R^2 reflect an adequate goodness of fit [49]. The R^2 explaining more than 80% of the variability in the maximum biomass production data for *C. braunii*. According to [50], biological systems, such as microalgal cultivation, exhibit natural variability in their processes and may present lower R^2 values (greater than 70%). Variability occurs due to factors that affect the development and functioning of microalgae, such as genetics and environmental

conditions (temperature, competition, resource availability, etc.).

As the calculated F-value for the lack of fit (2.163) was lower than the corresponding tabulated value (9.276), the error is not associated with a lack of fit of the model. Additionally, the calculated F-value for the regression (6.419) was higher than the tabulated F-value (4.387). Therefore, the model proposed by Eq. (10) is statistically significant and suitable for describing the data. As a result, the response surface and contour curves can be plotted, which are presented in Fig. 1a) and b), respectively.

By analyzing the response surface and contour curves, it can be observed that there is an optimal region, a maximum biomass concentration, in which the combination of saline concentrate percentage (20–60%) and urea concentration ($0.0\text{--}0.04 \text{ g L}^{-1}$) occurs. The optimal combination of factors for generating maximum biomass production within the experimental domain was 36.6% saline concentrate (63.4% brackish water) and 0.021 g L^{-1} urea concentration. Under the optimized conditions, $0.756 \pm 0.01 \text{ g L}^{-1}$ of biomass was obtained for *C. braunii* in 20 days of cultivation. According to [51], a concentration of 0.025 g L^{-1} of urea shows the highest growth rate, while concentrations above this value can induce stress on the microalga.

Considering the presence of an optimal region, experiment 7 (Exp 7), containing 50% saline concentrate, 50% brackish water, and no urea, was the chosen experiment for analysis as it presented the highest biomass production (0.556 g L^{-1}) using the highest percentage of saline concentrate. Although urea is directly associated with increased growth and biomass production in microalgae, cultivating without its addition promotes higher production of biomolecules, such as lipids [51, 52].

In cultivation systems, reduced urea consumption leads to lower operational costs, considering the average price of urea of approximately $\text{US\$}0.39 \text{ kg}^{-1}$. When scaled up, these savings become significant, particularly in processes using residual media, where the partial replacement of synthetic nutrients with alternative sources enhances the cost-effectiveness of cultivation and reduces dependence on commercial inputs, which account for about 27% of expenses in large-scale systems [53]. Moreover, lipid accumulation under nitrogen limitation contributes to lowering the cost per unit of biodiesel produced, given that the production cost of microalgal biodiesel is estimated at approximately $\text{US\$}3.90$ per liter [54].

In the present study, only the contribution of the salinity effect was sufficient to increase biomass production and, subsequently, the presence of biomolecules. Notably, the experiment with the optimal combination of factors produced a lower lipid content ($13.73 \pm 0.012\%$) than Experiment 7, which reached $29.40 \pm 1.64\%$.

Table 4 Analysis of variance for maximum biomass production

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	P-value
Regression	5	0.202	0.040	6.419	0.021
Residual error	6	0.037	0.006		
Lack-of-Fit	3	0.025	0.008	2.163	0.271
Pure error	3	0.011	0.003		
Total	11	0.228			
R^2 88.48%, R^2_{Adj} 69.67%, $F_{5,6;0.05}$ 4.387, $F_{3,3;0.05}$ 9.276					

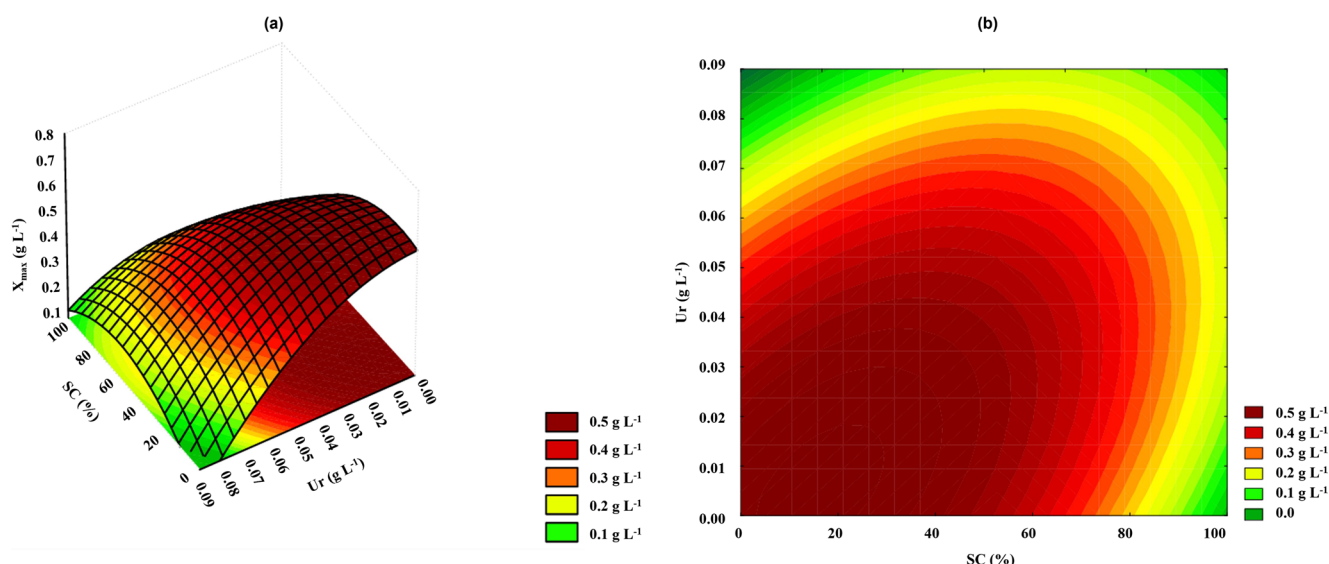


Fig. 1 Response surface and contour plots for the optimization of maximum biomass production of *Chlorolobion braunii* cultivated under varying concentrations of saline concentrate, brackish water, and urea. (a) 3D surface plot of saline concentrate percentage and urea concen-

tration vs. maximum biomass concentration. (b) Contour plot of saline concentrate percentage and urea concentration vs. maximum biomass concentration

Table 5 Medium composition and nutrient removal efficiency of a medium containing 50% saline concentrate and 50% brackish water (Exp 7) before and after *C. braunii* cultivation

Test	LQ*	Medium before cultivation (50% SC e 50% BW)	Medium after cultivation (supernatant Exp 7)	Removal Efficiency (%)
Salinity ($g\ L^{-1}$)	0.10	4.96	3.81	23.18
Phosphate (mg L^{-1})	0.25	1.20	<0.25	79.17
Bicarbonate Alkalinity (mg L^{-1})	2.0	45.45	17.70	61.05
Nitrates (mg L^{-1})	0.25	18.33	2.52	86.25

*Limit of quantification; SC saline concentrate, BW brackish water

Nutrient Removal Efficiency

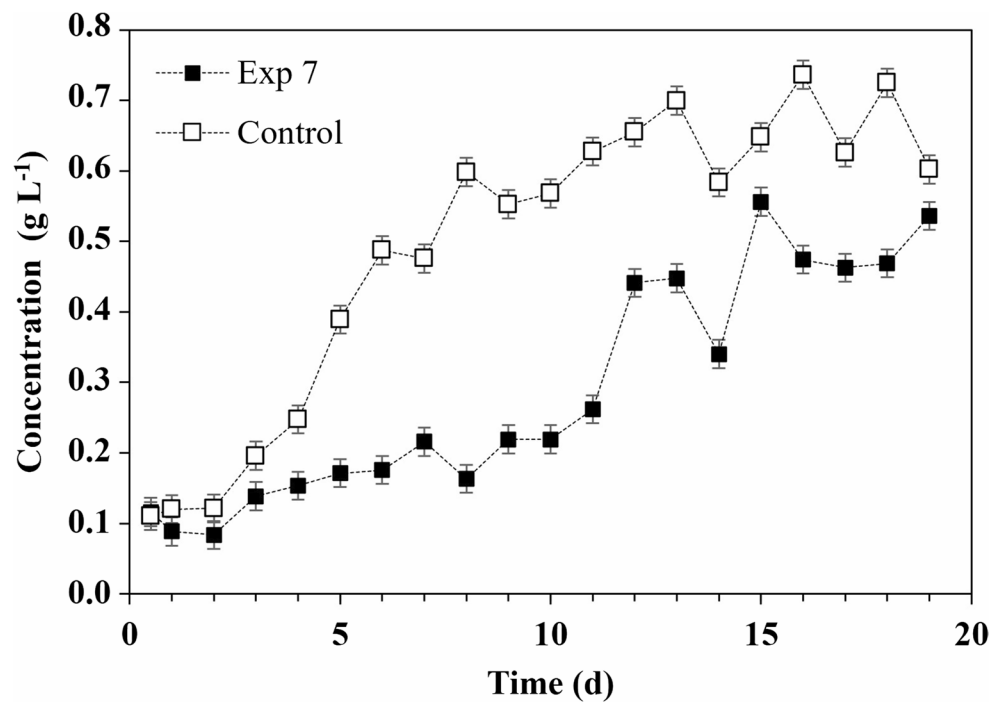
Table 5 presents the nutrient removal efficiency after *C. braunii* cultivation. The removal efficiency of inorganic nitrogen, in the form of nitrate, was 86.25%, while phosphorus had a removal efficiency greater than 79.17%. These reductions may be associated with the role of nitrogen and phosphorus in cellular metabolism. Nitrogen plays a key role in synthesizing proteins, lipids, and carbohydrates, while phosphorus is essential for algae growth, lipid production, fatty acid production, and metabolic processes, such as energy transfer and photosynthesis [55, 56]. observed a similar reduction with the cultivar *Tetradismus obliquus* GEEL-11 in municipal wastewater subjected to different salinity stress conditions. In that study, high nutrient

removal rates were recorded, ranging from 87 to 99% for nitrogen and 94–100% for phosphorus.

The medium with saline concentrate and brackish water showed a removal efficiency of 23.18% for salinity after *C. braunii* cultivation. According to [57, 58], the salinity removal mechanism by microalgae involves both bioadsorption and bioaccumulation. Bioadsorption, or the physical adhesion of salt to the cell surface, can occur through the substitution of other cations bound to the reactive groups on the algal cell surface by Na^+ , or a complexation between reactive groups such as carboxyl, hydroxyl, thiol, amino, phosphate, and hydroxyl-carboxyl, which adsorb salt ions through electrostatic attraction and/or covalent bonding [58]. On the other hand, bioaccumulation is a process that occurs inside the cells that is part of the metabolism, where salt ions enter the microalgal cell due to the ionic gradient between the algae and the external media [59].

The availability of essential nutrients directly influences osmoregulatory mechanisms and salt tolerance in microalgae, as nutrient scarcity may limit the uptake and excretion of saline ions [16]. Nitrogen limitation reduces the capacity for osmotic adjustment and resistance to salt stress, whereas phosphorus deficiency impairs energy (ATP) production, which is essential for the operation of ion transport systems [60, 61]. Moreover, adequate potassium levels help maintain membrane potential and ionic balance [62]. Therefore, a balanced supply of these nutrients can ensure that cells have sufficient energy to support the metabolic processes involved in active ion transport [63]. observed a similar reduction in salinity removal (23% reduction) when cultivating *Scenedesmus* sp., a green microalga, in Bold's Basal

Fig. 2 Growth curve of *C. braunii* cultivated in standard BG-11 medium (control) and with a combination of 50% saline concentrate and 50% brackish water (Exp 7) over 20 days



Medium (BBM) containing 2.5 g L⁻¹ NaCl for 20 days at 30 °C.

The bicarbonate alkalinity showed a reduction of 61.05%. The presence of bicarbonates in the medium increases nutrient uptake enzymes and promotes an increase in biomass quantity and the quality of its biochemical composition, with higher percentages of lipids and carbohydrates [64].

Kinetic Parameters of *C. braunii* Growth

It can be observed that both cultures showed an increase in biomass concentration over time, indicating the growth of *C. braunii*. Exp 7 and the control condition (*C. braunii* in standard BG-11 medium) showed an adaptation period (lag phase) of 3 days, as presented in Fig. 2. The need for an adaptation period may be related to the lack of pre-acclimatization to the cultivation conditions. Growth and biomass production are influenced by adapting the inoculum to the new cultivation environment [65]. The control showed an exponential phase lasting 14 days (3rd – 16th), with biomass concentration ranging from 0.195 to 0.736 g L⁻¹ until the 14th day. Meanwhile, Exp 7 had an exponential phase

lasting 13 days (3rd – 15th), where biomass concentration ranged from 0.138 to 0.556 g L⁻¹.

Table 6 presents the kinetic parameters of *C. braunii* growth in the Control medium (BG-11) and Exp 7. The specific growth rate ($\mu_{\max}$) of the control (0.137 d⁻¹) was higher than that of Exp 7 (0.117 d⁻¹), indicating that the medium containing saline concentrate and brackish water (Exp 7) exhibits slower growth compared to the standard medium (BG-11) [66]. cultivated the chlorophyte microalga *Monoraphidium neglectum* under different salinity conditions and observed similar behavior, with a reduction in $\mu_{\max}$ from 0.11 d⁻¹ for the highest salinity medium (0.2 M NaCl) compared to the standard BG-11 medium with $\mu_{\max}$ of 0.22 d⁻¹.

The control medium presented higher average productivity (0.035 ± 0.001 g L⁻¹ d⁻¹) and maximum production (0.736 ± 0.002 g L⁻¹) compared to Exp 7 (0.014 ± 0.002 g L⁻¹ d⁻¹ and 0.556 ± 0.001 g L⁻¹), as shown in Table 6. Salinity in the medium with saline concentrate and brackish water induces osmotic stress on the cell, resulting in reduced growth. However, despite the reduction in metabolism caused by salinity, *C. braunii* shows stress tolerance,

Table 6 Kinetic parameters and pH of *C. braunii* in standard BG-11 medium (control) and with a combination of 50% saline concentrate and 50% brackish water (Exp 7)

Sample	$\mu_{\max}$ (d ⁻¹)	Average productivity (g L ⁻¹ d ⁻¹)	Final production (g L ⁻¹)	Maximum production (g L ⁻¹)	Initial pH	Final pH
Control	0.137	0.035±0.001 ^a	0.462±0.002 ^a	0.736±0.002 ^a	8.63±0.01 ^a	8.90±0.01 ^a
Exp 7	0.117	0.014±0.002 ^b	0.409±0.002 ^a	0.556±0.001 ^b	8.25±0.01 ^b	8.65±0.01 ^b

Lowercase letters as superscripts within the same column for the same parameter indicate that the means are not statistically different at a 95% confidence level ($p > 0.05$)

demonstrating growth and biomass production. This characteristic is shared by other chlorophyte microalgae, such as *Chlamydomonas reinhardtii* cc124, *Chlorella* sp. MACC-360, *Coelastrrella* sp. MACC-549, and *Scenedesmus protuberans*, whose tolerance to salinity has also been highlighted by several authors [67–69]. These studies emphasize the adaptability of these species to saline environments, making them candidates for biotechnological systems under saline conditions.

Acclimation to salinity gradients can enhance microalgal tolerance to salt stress. Gradual exposure to increasing salt concentrations may enable their cells to activate physiological, metabolic, and molecular adaptive mechanisms, such as osmotic regulation, carbohydrate, and lipid accumulation as energy reserves, and the production of compatible metabolites [70, 71].

According to [72], microalgae under stress, such as salt stress, accumulate antioxidant enzymes to eliminate reactive oxygen species (ROS), including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione peroxidase (GPX), and glutathione reductase (GR). According to the authors, ROS detoxification can also occur through low molecular weight antioxidants (ascorbate, glutathione, and polyphenols) and the synthesis of compatible solutes, such as proline, glycine betaine, and other metabolites, which assist in osmotic adjustment. Acclimation processes can reduce the harmful effects of osmotic shock and promote the gradual adjustment of metabolic pathways, increasing protective mechanisms, such as compatible solute synthesis and ion homeostasis without sudden energy loss, potentially not compromising growth and biomass production [73].

The medium containing only saline concentrate and brackish water showed a pH value similar to the control,

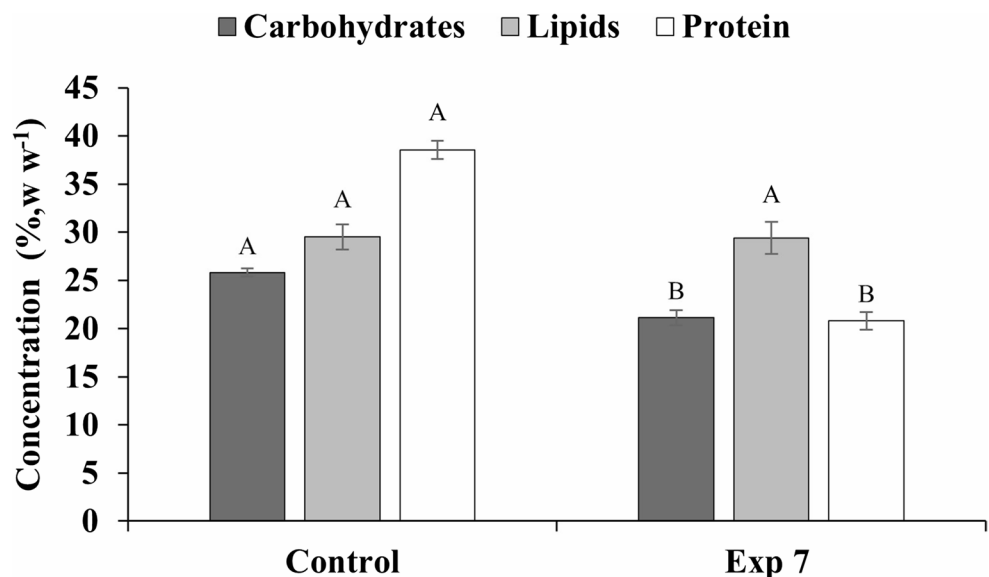
which is favorable for microalgae growth, indicating that this medium can maintain ideal conditions for its development. A slight increase in pH was observed in both the control (8.63 ± 0.01 – 8.90 ± 0.01) and Exp 7 (8.25 ± 0.01 – 8.65 ± 0.01) after twenty-one days of treatment with the microalgae. This increase may be linked to the uptake of dissolved carbon dioxide in the growth medium during the algae's photosynthesis [69]. A similar increase (7.76 – 8.56) was observed by [29] when cultivating *C. braunii* in dairy wastewater for ten days.

Biochemical Composition of *C. braunii* Biomass

The biochemical composition of carbohydrates, lipids, and proteins of *C. braunii* biomass for the Control and Exp 7 conditions is presented in Fig. 3. The protein composition for Exp 7 showed a 45.9% reduction compared to the control (from 38.5% to 20.8%). This reduction suggests that the stress caused by the salinity of the Exp 7 medium affected protein synthesis. Saline stress also increases the generation of reactive oxygen species (ROS), triggering oxidative stress, which causes damage to DNA, lipids, and proteins, leading to metabolic dysfunction and potentially causing cell death [44].

According to [74], under stress conditions such as salinity, microalgae produce reactive oxygen species (ROS) for instance hydrogen peroxide (H_2O_2), superoxide radical (O_2^-), and hydroxide ion (OH^-), among others. These species are toxic and damage proteins, nucleic acids, and other cellular macromolecules, inhibiting growth and leading to cell death [75]. observed a reduction in protein composition by 27.6% for *Desmodesmus* sp. YT cultivated in a medium with 16‰ salinity for 10 days (296.81 mg g^{-1}) compared to 0‰ salinity (395.80 mg g^{-1}).

Fig. 3 Concentration of carbohydrates, proteins, and lipids on a dry weight basis (% w w⁻¹) for the biomass of *C. braunii* in standard BG-11 medium (control) and with 50% saline concentrate and 50% brackish water (Exp 7). A, B Identical uppercase letters for the same parameter indicate that the means do not differ statistically at a 95% confidence level between the cultures ($p > 0.05$); The results of the biomolecule concentrations are from the last day of cultivation



The carbohydrate composition is higher for the control culture ($25.80 \pm 0.45\%$) than for Exp 7 ($21.14 \pm 0.80\%$). However, it is noted that the reduction in carbohydrate percentage was low, remaining below 30% in both situations. Lipid synthesis did not change, and the lipid percentage for the control medium ($29.49 \pm 1.33\%$) was similar to that of Exp 7 ($29.40 \pm 1.64\%$). Lipids and carbohydrates are the preferred forms of energy storage in microalgae under various stress conditions due to their hydrophobic nature and reduced states. These compounds are used by the cells in adverse conditions to ensure their survival [76].

According to [74], the increase in lipid synthesis and the maintenance of carbohydrate levels are related to energy storage triggered by saline stress. According to the authors, these processes occur in parallel, allowing carbohydrates to be stored and excess to be converted into lipids. For [77], lipid synthesis under saline stress is mainly mediated by an acetyl-CoA-dependent Kennedy pathway, essentially a side effect of ROS detoxification, causing excess energy and reductants to participate in lipid accumulation [46]. found a similar composition when cultivating *Desmodesmus* sp.KNUA024, a green microalga, in saline concentrate with added phosphorus, in an indoor culture, obtaining $18.66 \pm 0.94\%$ carbohydrates and $27.11 \pm 0.95\%$ lipids.

Fatty acid Composition (FAME)

Table 7 shows the fatty acid profile of *C. braunii* cultivated in BG-11 medium (Control) and 50% saline concentrate with 50% brackish water (Exp 7). The total concentration of

Table 7 Fatty acid profile of *C. braunii* grown in 50% concentrate saline and 50% brackish water

Fatty acid	Nomenclature		Treatments	
			Control	Exp 7
C14:0	Miristic acid	3.64 ± 1.31	-	
C14:1	Myristoleic acid	2.15 ± 0.64	-	
C16:0	Palmitic acid	20.07 ± 1.09	24.94 ± 1.78	
C16:1	Palmitoleic acid	-	1.96 ± 0.17	
C17:0	Margaric acid	3.13 ± 0.23	2.63 ± 0.25	
C17:1	Heptadecanoic acid	3.95 ± 0.51	3.71 ± 0.38	
C18:0	Stearic acid	11.32 ± 2.36	2.38 ± 0.28	
C18:1n9t	Elaidic acid	1.26 ± 0.22	-	
C18:1n9c	Oleic acid	9.21 ± 0.50	38.44 ± 0.55	
C18:3n6	γ -linolenic acid	15.87 ± 0.36	11.55 ± 0.29	
C18:3n3	α -linolenic acid	25.65 ± 0.19	12.85 ± 1.06	
C20:0	Arachidic acid	3.77 ± 0.18	1.54 ± 1.87	
Σ saturated		41.91 ± 5.19^a	31.49 ± 2.79^a	
Σ monounsaturated		16.56 ± 1.88^a	44.11 ± 1.11^b	
Σ polyunsaturated		41.53 ± 0.55^a	24.40 ± 1.35^b	

Lowercase superscript letters within the same parameter indicate that the means do not differ statistically at the 95% confidence level ($p > 0.05$); The results are presented as mean $\pm$ standard deviation of the fatty acid concentration in the microalgal biomass on the last day of cultivation

saturated fatty acids (Σ SFA) showed no statistical difference between the control and Exp 7. The total concentration of monounsaturated fatty acids (MUFA) significantly increased in Exp 7 (44.11%) compared to the control (16.56%). Meanwhile, the total concentration of polyunsaturated fatty acids (PUFA) was lower in Exp 7 (24.40%) compared to the control (41.53%). The increase in MUFA and the reduction in PUFA may be associated with the generation of reactive oxygen species (ROS) under aerobic conditions, resulting in lipid peroxidation, especially of polyunsaturated fatty acids. Under stress conditions, microalgal cells can activate a series of antioxidant defenses and modify their lipid profile in response to oxidative stress [78].

In the presence of saline stress, the MPC transporter (mitochondrial pyruvate carrier 2) was positively regulated to increase the efficiency of pyruvate transport in the malate/pyruvate cycle. Subsequently, pyruvate was efficiently converted into acetyl-CoA [77]. According to the authors, fatty acids with C16 and C18 chains are commonly synthesized due to the action of acetyl-CoA (acetyl coenzyme A), which drives fatty acid production through the enzyme acetyl-CoA carboxylase. This enzyme converts bicarbonate and acetyl-CoA into malonyl-CoA, a process catalyzed by the malonyl-acetyl protein [77, 79]. In this study, palmitic acid (C16:0) increased by 24.26% in Exp 7 (24.94%) compared to the control (20.07%).

On the other hand, stearic acid (C18:0) showed a 78.90% decrease from the control (11.32%) to Exp 7 (2.36%). Oleic acid (C18:1n9c) exhibited a significant increase (higher than 100%) in concentration in Exp 7 (38.44%) compared to the control (9.21%). Gamma-linolenic acid (C18:3n6) and alpha-linolenic acid (C18:3n3) showed a decrease of 27.22% and 49.90%, respectively, from the control to Exp 7. The reduction in the concentrations of C18:0, C18:3n6, and C18:3n3 may be associated with stress conditions, such as salinity or nutrient restriction, which suppress the activity of enzymes in the cell that produce PUFAs (such as FADS12 and FADS15) and increase the production of enzymes responsible for monounsaturated fatty acids, such as $\Delta 9$ -desaturase, which converts C18:0 into C18:1n9c [80]. According to [81], to prevent the formation of reactive oxygen species under stress, C18:0 is converted into C18:1n9c using $\Delta 9$ desaturase by adding a double bond. This desaturation is caused by an oxidative process that requires O_2 , NADH, NADPH, and substrate.

The changes observed in the fatty acid profile of Exp 7, characterized by an increase in the fraction of monounsaturated fatty acids and a reduction in polyunsaturated fatty acids compared with the control, indicate a simultaneous improvement in the oxidative stability and cold-flow properties of the biodiesel, bringing it closer to international quality standards. According to [82], higher levels of

unsaturation result in higher oxidation rates; thus, polyunsaturated fatty acids oxidize more readily due to the lower dissociation energy of allylic hydrogen, whereas saturated and monounsaturated fatty acids exhibit greater stability. According to these authors, oxidative stability follows the order C18:0 $\gg$ C18:1 $\gg$ C18:2 $>$ C18:3, indicating that oils with lower degrees of unsaturation have greater resistance to oxidation. This trend is observed in the fatty acid profile obtained in Exp 7, as seen by a significant reduction in the polyunsaturated fraction, particularly C18:3 (41% reduction), and an increase in monounsaturated fatty acids, predominantly oleic acid, indicating greater resistance to oxidation.

The presence of saturated fatty acids, mainly C16:0, together with a fraction of monounsaturated fatty acids, especially C18:1 (as observed in Exp 7) indicates favorable cold-flow properties. These two fatty acids jointly promote a controlled nucleation process, resulting in the formation of smaller, less interconnected crystals, which improves biodiesel fluidity without compromising stability at low temperatures. According to [83], saturated esters contribute to controlled nucleation processes due to their linear structure and higher melting point, acting as nucleation centers during biodiesel cooling, whereas unsaturated esters, which have lower melting points and surfactant-like behavior, inhibit crystal growth and aggregation.

The profile of Exp 7, as shown in Table 7, with the highest percentages of C16 and C18, is considered ideal for biodiesel production. According to [42], the carbon chains in these fatty acids offer a balance between energy density and fuel fluidity.

Evaluation of Biodiesel Properties

Table 8 presents the quality parameters of the theoretical biodiesel produced from the microalga *C. braunii*, comparing them with the reference values from standards such as

Table 8 Quality parameters of theoretical biodiesel from the microalga *C. braunii*

Properties	Standards			<i>C. braunii</i>	
	ASTM D6751	EN 14,214	ANP N°968/24	Control	Exp 7
Cetane number	≥ 47	≥ 51	≥ 45	41	48
Iodine value (g Iodine 100 g ⁻¹)	≤ 120	-	-	141.8	112.0
Saponification value	-	-	-	206.3	204.8
CFPP (°C)	-5 a -15	-	19	-1.4	-11.3
LCSF (% weight)	-	-	-	4.79	1.65
DU (% weight)	-	-	-	99.6	92.9

CFPP Cold filter plugging point, LCSF Long-chain saturated factor, DU Degree of unsaturation

ASTM D6751 (USA), EN 14,214 (Europe), and ANP n° 968/24 (Brazil) [84].

The control showed a cetane number (43) below the reference values, indicating lower ignition quality. On the other hand, Exp 7 reached the values of ASTM and ANP with a cetane number of 48. However, this value does not meet the EN limit. The cetane number indicates a fuel ignition quality, reflecting the speed at which the fuel ignites after compression in the engine [85, 86]. A similar cetane number value (47.77) was reported by [31] when *C. braunii* was cultivated in an industrial effluent, reflecting the higher content of saturated fatty acids in this strain.

The EN 14,214 standard requires a cetane number (≥ 51) higher than that required by ASTM D6751 (≥ 47) and ANP (≥ 48). This stricter requirement in Europe may be related to the fact that Europe has several regions with cold climates, where fuels with a lower cetane number may have difficulty starting the engine. According to [87], a high cetane number ensures good engine start-up at low temperature and pressure, in addition to causing the fuel to burn before there is adequate mixing between the fuel and air.

The iodine value (IV) is determined by the amount of iodine (I₂) that reacts with carbon-carbon double bonds, thus being directly proportional to the degree of unsaturation of FAME [85]. The control had an iodine value above the limit (141.8), indicating higher unsaturation. Meanwhile, Exp 7 had an IV of 112.0 within the limit, suggesting lower unsaturation. The saponification value, which reflects the complete saponification of fatty acids in the biodiesel, was 204.8 for *Chlorolobion braunii* in Exp 7, a result similar to that of the control (206.3). This range is comparable to that of other strains, such as *Chlorella vulgaris*, which showed an average value of 210.96 [86].

The cold filter plugging point (CFPP) represents the minimum temperature at which biodiesel can be used without crystallization, acting as a measure of performance in cold climates [85, 88]. The control had a CFPP of -1.4 °C, outside the limit, indicating lower efficiency in low temperatures. However, Exp 7 had a CFPP of -11.3 °C, within the limit, indicating a better performance in cold climates. A similar behavior was reported by [31], where *C. braunii* showed a CFPP value (12.78 °C) compatible with the limits established by the ANP when cultivated in industrial effluents.

Exp 7 showed a decline in the degree of unsaturation (92.9) compared to the control (99.6). This decrease in the degree of unsaturation enhances the oxidative stability of biodiesel. These changes might be related to differences in the fatty acid profile, where a higher presence of C18:1n9c was observed in Exp 7. Oxidative stability is closely linked to the degree of unsaturation. In general, the higher the unsaturation, the lower the oxidative stability, although the

autoxidation rate of unsaturated fatty acids varies according to the number and position of the double bonds [89, 90].

Modifying factors such as temperature and light intensity during cultivation can improve oxidative stability by increasing the proportion of saturated fatty acids, such as palmitic acid (C16:0), during cultivation. According to [91], the fatty acid composition changes with light intensity. According to the authors, by increasing irradiance, the amount of C16–18 fatty acids increased. This rise results from light-induced changes in pH and NADPH in the fluid matrix located within chloroplasts, where a significant part of photosynthesis occurs, which influences and modulates the activity of key enzymes in the fatty acid biosynthesis process [91]. According to [92], SFA and MUFA increase with decreasing temperatures due to limited nitrogen absorption in the medium induced by low temperatures, as both nitrate consumption and the reduction of nitrate to ammonia in cells are known to be temperature-sensitive.

Practical Implications and Scalability

Implementing a pilot-scale microalgae cultivation system integrated with effluent treatment, such as the cultivation of *Chlorolobion braunii* using saline concentrate, is a sustainable approach to resource recovery and bioproduct generation. Implementation should be carried out gradually, beginning with laboratory-scale optimization, followed by operational validation and subsequent transition to pilot scale, where system stability, productivity, and technical and economic performance under real operating conditions are evaluated. This validation will help overcome scale-up challenges, such as kinetic limitations and variations in light and nutrient gradients; factors that constrain growth rates and system productivity and that often compromise stability and reproducibility at larger volumes.

During the laboratory-scale optimization stage, following the initial batch phase, used for growth characterization and determination of the optimal concentration (as conducted in the present study), the cultivation of *C. braunii* in saline concentrate and brackish water should operate in a semi-continuous mode. This involves the periodic removal of a fraction of the reactor volume, followed by replenishment with renewed wastewater, to maintain biomass concentration at the optimized level [93]. The semi-continuous system allows evaluation of the culture's response to continuous nutrient replenishment and the potential accumulation of metabolites, providing technical support for adjusting operational parameters prior to scale-up. In addition, semi-continuous systems tend to exhibit lower biomass production costs than batch systems (US\$1,380–2,040 per ton), typically ranging from US\$1,130 to US\$1,200 per ton of biomass produced [94].

Integrating a pilot system operating under semi-continuous cultivation of *C. braunii* with a desalination plant could be achieved by installing an independent unit supplied with a fraction of the brackish water and the saline concentrate generated after reverse osmosis. The produced biomass could be directed both toward biodiesel production and the extraction of higher-value biomolecules, such as proteins, pigments, and exopolysaccharides, enabling the evaluation of different bioproduct valorization pathways. These compounds may contribute to reducing overall process costs. According to [54], the co-production of pigments using wastewater in microalgae cultivation can reduce the production cost of microalgal biodiesel from US\$3.90 to approximately US\$0.54 per liter.

Although no real-world examples of integration between desalination plants and microalgae cultivation systems currently exist, theoretical and modeling studies have indicated the feasibility of this approach, particularly when algal cultivation is used as a route for CO₂ capture. Based on modeling analyses [95], reported that integrating microalgae cultivation focused on CO₂ capture enables the simultaneous utilization of residual streams, such as flue gases, waste heat, and brines, thereby promoting gains in both energy efficiency and rational water use. Thus, although experimental validation is still required, the available evidence in the literature suggests that such integration is technically feasible and could contribute to more sustainable desalination systems. Consequently, pursuing this integration in future studies represents a key direction for structuring and practically implementing the association of desalination systems with microalgae cultivation for biomolecule production.

Conclusion

This pioneering study demonstrates that cultivating *Chlorolobion braunii* in saline effluents offers a promising alternative for biofuel and high-value bioproduct generation. The approach successfully valorizes desalination waste while integrating bioremediation with circular bioeconomy principles - all without requiring pretreatment or synthetic media supplementation. The saline concentrate application effectively supported both biomass production and effluent bioremediation, despite showing lower kinetic parameters than control cultures. Particularly noteworthy was the 50% saline concentrate treatment (Exp 7), which achieved high biomass productivity (0.556 g L⁻¹), elevated lipid (29.40%) and carbohydrate (21.14%) content, with significant removal of nitrates (86.25%), phosphates (79.17%), and salinity (23.18%). The resulting biomass showed a lipid profile compatible with biodiesel production requirements established by ASTM D6751 and ANP standards.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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