BRACKISH GROUNDWATER AS A LOW-COST CULTIVATION MEDIUM FOR *Spirulina* sp. LEB 18: BIOMOLECULE PRODUCTIVITY AND COST ASSESSMENT

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Abstract: Spirulina sp. LEB 18 was cultivated in Zarrouk medium and brackish groundwater (BW) supplemented with 50% of Zarrouk nutrient concentrations, combined with three LED spectra (white, blue, and white/blue). Biomass concentration, biomolecule productivity, and production costs were evaluated. Zarrouk medium under white LED maximized biomass concentration (3.12 g L^{-1}) and protein productivity (170.00 mg L^{-1} d^{-1}). BW sustained the highest carbohydrate productivity (122.09 mg L^{-1} d^{-1}), exceeding Zarrouk-based treatments under the same spectrum. Cost analysis revealed that BW-based treatments consistently reduced biomass and carbohydrate production costs, indicating a preliminary reagent-cost advantage alongside compositional flexibility for biotechnological applications.

Palavras-chave: *Arthrospira*; light spectrum; microalgae; sustainable biorefinery.

INTRODUCTION

Microalgae are versatile microorganisms capable of synthesizing high-value proteins, carbohydrates, lipids, and pigments from CO_2 and solar energy (Sun et al., 2018). Among them, *Spirulina* sp. (*Arthrospira* sp.) stands out for its high protein content (~60% d.w.), digestibility, and tolerance to alkaline and saline conditions (Borowitzka, 2018; Morais et al., 2008). These characteristics make this cyanobacterium a promising candidate for cultivation in alternative media, reducing freshwater consumption in large-scale processes.

Brackish groundwater (BW), abundant in semi-arid regions of Brazil, presents moderate salinity and dissolved ions that can partially meet the mineral demands of microalgae (Bezerra et al., 2022). However, the osmotic stress imposed by saline environments reorganizes cellular metabolism, generally shifting carbon partitioning from proteins toward compatible solutes involved in osmoregulation (Markou et al., 2021).

This behavior, which may also be influenced by the reduced nutrient supplementation associated with BW use, can be exploited for the directed production of bioactive carbohydrates. Importantly, the use of BW also reduces dependence on costly synthetic media, potentially lowering the overall production cost of microalgal biomass and derived biomolecules.

Light-emitting diodes (LEDs) enable precise and energy-efficient spectral control, modulating photosynthesis and metabolic pathways (Mahari et al., 2024). Blue light tends to favor metabolic regulation and pigment synthesis, while combined spectra can balance photochemical efficiency and biomolecule accumulation (Prates et al., 2020). Nevertheless, the interaction between spectral quality and salinity stress remains insufficiently explored for *Spirulina*.

This study investigated the combined effects of BW and different LED spectra on growth, biochemical productivity, and production costs of *Spirulina* sp. LEB 18, with emphasis on the metabolic modulation potential for sustainable biorefinery applications.

MATERIAL AND METHODS

Microorganism and cultivation conditions. *Spirulina* sp. LEB 18 (Morais et al., 2008) was cultivated in 1 L Erlenmeyer photobioreactors (0.8 L working volume) at 30°C with continuous aeration (0.1 vvm). Six treatments were conducted in duplicate for 15 days, with conventional Zarrouk or BW supplemented with 50% of C, N, P, Fe, and chelating agent concentrations from Zarrouk medium (Bezerra et al., 2022). All treatments received a 12 h light/12 h dark photoperiod with an equal total daily photon dose; cultivation used three LED spectra, white, blue, and combined white/blue (6 h white + 6 h blue, alternated within the same 12 h light period), at 41.6 μmol photons m^{-2} s^{-1}.

Analytical determinations. Biomass concentration was monitored by optical density at 670 nm and converted to dry biomass concentration using a previously established calibration curve. At the end of cultivation, the biomass was harvested and analyzed for biochemical composition. Protein content was determined according to Lowry et al. (1951), carbohydrate content according to Dubois et al. (1956), and lipid content according to Folch et al. (1957). The results were expressed as percentage of dry biomass.

Biomass and biomolecule productivity. Maximum biomass concentration (X_{max}) was obtained from the growth curves. Biomass productivity (P_X) was calculated as the ratio between biomass concentration and cultivation time and expressed as mg L^{-1} d^{-1}. The final concentration of each biomolecule was calculated by multiplying X_{max} by the respective biochemical fraction in the biomass. Protein, lipid, and carbohydrate productivities were calculated by multiplying biomass productivity by the respective biochemical fractions and expressed as mg L^{-1} d^{-1}.

Cost analysis. Reagent costs for Zarrouk medium were obtained in June 2026 from the Sigma-Aldrich Brazil manufacturer's website, corresponding to USD 3.19 L^{-1}. The BW-based medium was estimated at USD 1.37 L^{-1}, considering only the supplemented nutrients, since the brackish groundwater itself was assumed to be freely available. The medium-related production cost of biomass was calculated by dividing the culture medium cost by the final biomass concentration. The medium-related production cost of each biomolecule fraction was calculated by dividing the culture medium cost by the final concentration of the respective biomolecules, obtained from X_{max} and biochemical composition. Costs were expressed as USD g^{-1}, adapted from Park et al. (2013).

Statistical analysis. Results were analyzed by analysis of variance (ANOVA) followed by Tukey's test at a 95% confidence level ($p<0.05$).

RESULTS AND DISCUSSION

Biomass concentration and productivity. Zarrouk medium under white LED yielded the highest X_{max} (3.12 ± 0.14 g L^{-1}) and productivity (382.88±15.54 mg L^{-1} d^{-1}), significantly higher ($p < 0.05$) than all other treatments (Table 1). This outcome is consistent with the broad spectral coverage of white LED, which simultaneously activates chlorophyll *a* (430–680 nm) and phycobiliprotein absorption bands (550–620 nm), sustaining efficient photosynthetic carbon fixation under nitrogen-replete conditions (Bhat et al., 2023; Prates et al., 2018). In contrast, BW-based treatments yielded X_{max} values of 1.54–2.00 g L^{-1}, which differed significantly ($p < 0.05$) among themselves, but were all significantly lower ($p < 0.05$) than the Zarrouk control.

Table 1. Maximum of biomass concentration (X_{max}) and productivity (P_{max}) of *Spirulina* sp. LEB 18 cultivated under different culture media and LED spectra.

Assay	X_{max} (g L^{-1})	P_{max} (mg L^{-1} d^{-1})
1	$3.12±0.14^{a}$	$382.88±15.54^{a}$
2	$2.00±0.16^{c}$	$274.04±11.29^{b}$
3	$1.89±0.17^{cd}$	$134.13±10.26^{d}$
4	$1.93±0.19^{c}$	$114.71±3.47^{e}$
5	$2.42±0.16^{b}$	$163.04±8.21^{c}$
6	$1.54±0.11^{d}$	$241.76±29.72^{b}$

Means followed by different superscript lowercase letters in the same column differ significantly (ANOVA + Tukey, p < 0.05). 1: White LED Zarrouk. 2: White LED brackish groundwater (50%). 3: Blue LED Zarrouk. 4: Blue LED brackish groundwater (50%). 5: White/Blue LED Zarrouk. 6: White/Blue LED brackish groundwater (50%).

This growth constraint is attributable to the elevated Na^{+} and Mg^{2+} concentrations in BW, which impose ionic stress and require energy reallocation toward osmotic homeostasis rather than biomass accumulation, as documented by Bezerra et al. (2022) for the same strain cultivated in 100% brackish groundwater without nutrient supplementation (X_{max} = 1.22 g L^{-1}). Notably, despite lower biomass concentrations, BW/white LED sustained the highest carbohydrate productivity (122.09 ± 9.65 mg L^{-1} d^{-1}), higher than ($p < 0.05$) Zarrouk-based treatments under the same spectrum (98.83 ± 1.80 mg L^{-1} d^{-1}).

The culture medium exerted a dominant and statistically significant ($p < 0.05$) effect on biomolecule productivity (Table 2). Protein productivity was highest in Zarrouk/white LED (170.00 ± 1.25 mg L^{-1} d^{-1}), consistent with findings by Prates et al. (2018), who reported high protein yields for Spirulina sp. LEB 18 under white LED in Zarrouk medium. Under BW, protein productivity declined significantly ($p < 0.05$) across all spectra (15.59–48.80 mg L^{-1} d^{-1}). This reduction may reflect both the inhibitory effect of high ionic strength on nitrogen assimilation, with the associated energetic cost of Na^{+} extrusion (Bezerra et al., 2022; Duarte et al., 2020), and the lower nitrogen availability inherent to the 50% nutrient supplementation used in BW treatments, which limits direct attribution of the response to osmotic stress alone.

Table 2. Biochemical productivity of *Spirulina* sp. LEB 18 cultivated under different culture media and LED spectra.

Assay	$P_{Proteins}$ (mg L^{-1} d^{-1})	P_{Lipids} (mg L^{-1} d^{-1})	$P_{Carbohydrates}$ (mg L^{-1} d^{-1})
1	$170,00±1,25^{a}$	$73,28±6,31^{a}$	$98,83±1,80^{b}$

2	31,48±0,84^{e}	43,35±1,38^{c}	122,09±9,65^{a}
3	45,52±0,78^{c}	21,43±0,92^{e}	34,71±0,73^{e}
4	15,59±0,82^{f}	17,93 ± 0,78^{f}	48,98±1,88^{d}
5	42,37±1,20^{d}	31,91±4,37^{d}	25,46±0,78^{f}
6	48,80 ± 8,38^{b}	37,80 ± 3,98^{b}	70,76 ± 0,49^{c}

Means followed by different superscript lowercase letters in the same column differ significantly (ANOVA + Tukey, p < 0.05). 1: White LED Zarrouk. 2: White LED brackish groundwater (50%). 3: Blue LED Zarrouk. 4: Blue LED brackish groundwater (50%). 5: White/Blue LED Zarrouk. 6: White/Blue LED brackish groundwater (50%).

Protein productivity varied with LED spectrum within each medium, with patterns differing between Zarrouk and BW conditions, an effect broadly consistent with reports that short-wavelength radiation upregulates phycobilisome synthesis in cyanobacteria, as observed for multiple *Arthrospira* strains by Milia et al. (2022).

Carbohydrate productivity was significantly higher ($p < 0.05$) under BW/white LED (122.09 ± 9.65 mg L^{-1} d^{-1}) than under the equivalent Zarrouk treatment (98.83 ± 1.80 mg L^{-1} d^{-1}). This pattern is consistent with reports by Duarte et al. (2020), who observed substantial increases in carbohydrate content in *Spirulina* sp. LEB 18 cultivated with BW and reduced Zarrouk supplementation, an effect generally attributed to osmotic stress-induced synthesis of low-molecular-weight compatible solutes (Bezerra et al., 2022), though the concurrent reduction in nutrient supply may also have contributed to this carbon reallocation. Lipid productivity did not show a consistent medium-driven pattern, suggesting that lipid metabolism responded differently to the combined BW and spectral conditions than the protein–carbohydrate balance.

BW-based treatments presented significantly lower ($p < 0.05$) biomass production costs than Zarrouk conditions across all LED spectra (Table 3), with values ranging from USD 0.69 ± 0.05 to 0.89 ± 0.06 g^{-1} vs. USD 1.02 ± 0.05 to 1.70 ± 0.15 g^{-1}, directly reflecting the difference in medium cost (USD 1.37 L^{-1} for BW vs. USD 3.19 L^{-1} for Zarrouk). This finding aligns with Bezerra et al. (2022), who highlighted that substituting synthetic nutrients with BW can substantially reduce cultivation costs while maintaining appreciable biomass outputs, particularly relevant to large-scale operations where medium cost can represent up to 35% of total production expenses.

Table 3. Production costs (USD g^{-1}) of biomass and biomolecule fractions of *Spirulina* sp. LEB 18 under different culture media and LED spectra.

Assay	Biomass (USD g^{-1})	Protein (USD g^{-1})	Lipids (USD g^{-1})	Carbohydrates (USD g^{-1})
1	1.02±0.05^{c}	2.30±0.11^{d}	5.38±0.68^{c}	3.96±0.11^{c}
2	0.69±0.05^{d}	5.98±0.59^{a}	4.34±0.18^{d}	1.49±0.09^{e}
3	1.70±0.15^{a}	5.03±0.51^{b}	10.69±1.37^{a}	6.57±0.48^{b}
4	0.72±0.07^{d}	5.33±0.61^{a}	4.62±0.34^{cd}	1.69±0.11^{e}
5	1.32±0.09^{b}	5.09±0.30^{b}	6.86±1.20^{b}	8.46±0.44^{a}
6	0.89±0.06^{d}	4.48±0.71^{c}	5.71±0.44^{c}	3.04±0.19^{d}

Means followed by different superscript lowercase letters in the same column differ significantly (ANOVA + Tukey, p < 0.05). 1: White LED Zarrouk. 2: White LED brackish groundwater (50%). 3: Blue LED Zarrouk. 4: Blue LED brackish groundwater (50%). 5: White/Blue LED Zarrouk. 6: White/Blue LED brackish groundwater (50%).

For protein, Zarrouk/white LED achieved the lowest cost (USD 2.30 ± 0.11 g^{-1}), significantly lower ($p < 0.05$) than all other treatments, driven by its superior protein yield rather than medium price. BW treatments showed protein costs of USD 4.48–6.00 g^{-1}, reflecting the combined effect of reduced medium cost and substantially lower protein accumulation. This trade-off illustrates that protein cost can be determined by medium price and biomolecule yield: reducing medium cost alone does not offset a proportionally larger decline in protein accumulation. For carbohydrates, the pattern was reversed, BW/white LED (USD 1.49 ± 0.09 g^{-1}) and BW/blue LED (USD 1.91 ± 0.05 g^{-1}) presented the two lowest costs, both significantly lower ($p < 0.05$) than their Zarrouk counterparts (USD 3.96 ± 0.11 and 6.57 ± 0.48 g^{-1}, respectively), because here the osmotic stress-driven carbohydrate surplus offsets the penalty of lower biomass.

These results show BW as a strategically versatile medium: economically favourable for total biomass production across all conditions, and specifically advantageous for carbohydrate-oriented biorefinery streams where salinity stress acts as a productive metabolic trigger rather than a constraint.

CONCLUSION

The results suggest that culture medium composition had a stronger influence on biomolecule productivity than LED spectrum under the evaluated conditions. Zarrouk medium under white LED yielded the highest protein productivity and the lowest protein production cost (USD 2.30 g^{-1}), the preferred condition for protein-oriented applications. BW reduced production costs specifically for biomass and carbohydrates across all LED spectra, with BW/white LED achieving the lowest carbohydrate cost (USD 1.49 g^{-1}); for protein, the lowest cost remained with Zarrouk/white LED. These results indicate BW as a low-cost medium for biomass and carbohydrate-oriented production, with potential as a strategic input for sustainable microalgal biorefineries.

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