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SEAWATER EMPLOYED IN *Spirulina* CULTIVATION TO REPLACE FRESHWATER AND NUTRIENTS

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ABSTRACT

The number of people in the world without access to freshwater and food is increasing, leading to public health problems. Microalgae offer promising solutions for reducing freshwater usage, reusing nutrients, and producing renewable biomass. Renowned for their robustness in large-scale outdoor cultivation to biomass production, *Spirulina* strains boast a high protein content and of nutritional benefits. To reduce operational costs with microalgae biomass production, seawater (SW), rich in essential elements, can be used in cultivation. This study explored the cultivation of *Spirulina* sp. LEB 18 in SW diluted with distilled water under outdoor conditions. The highest results for maximum biomass concentration were obtained with 25% and 50% v v^{-1} SW. In the three lower concentrations of SW, the biomass productivity was higher than that obtained with the highest concentration of SW. In the treatment with 75% v v^{-1} SW, compared to the standard culture in the literature, kinetic results showed ~34% and ~10% higher maximum biomass concentration and biomass productivity, respectively, relative to the use of freshwater. Therefore, the potential of using SW in *Spirulina* cultivation to efficiently produce biomass while reducing freshwater usage was highlighted.

Keywords: Biomass. Freshwater Scarcity. Microalgae Cultivation. Nutrient Recovery. Seawater Utilization.

1 INTRODUCTION

Worldwide, 1.8 billion people live in households without freshwater supply. In Brazil, about 35 million people live without access to freshwater, exacerbating public health problems and quality of life.[1] Microalgae can be employed to alleviate freshwater scarcity while contributing to nutrient recovery and the production of renewable biomass with diverse applications. The genus *Spirulina* has been promisingly explored for food enrichment due to its high protein concentration (~60% w w^{-1}) and its nutritional and therapeutic properties.[2]

The levels of freshwater and nutrients used for microalgae cultivation represent up to 35% of operational costs.[3] Therefore, strategies have been developed to use alternative water sources and reduce nutrient demand.[4] Seawater (SW), despite its high salinity (~35 g L^{-1}), contains favorable chemical elements for microalgal metabolism, such as sodium chloride, carbon, magnesium, calcium, and iron.[5] Cultivating *Spirulina* in SW has shown promise for producing useful biomolecules in the food industry, but it requires dilution with freshwater and/or pretreatment.[6] Thus, this study aimed to produce *Spirulina* biomass using SW in outdoor conditions, in a cultivation system with minimized costs.

2 MATERIAL & METHODS

Strain, Culture Media and Culture Conditions

The strain used was *Spirulina* sp. LEB 18[7], which belongs to the Strain Bank of the Laboratory of Biochemical Engineering (LEB) at the Federal University of Rio Grande (FURG). The inoculum used was propagated in Zarrouk medium[8] in 500 L open raceway bioreactors kept in a greenhouse with transparent film under outdoor conditions (uncontrolled illuminance and temperature). The SW was collected at the "Marine Aquaculture Station Prof. Marcos Alberto Marchiori (EMA)", belonging to the Institute of Oceanography at FURG. After collection, the SW was filtered using qualitative filter paper to eliminate particulates, followed by refrigeration until characterization and use in the assays. The cultures were conducted in duplicate, in batch mode, using SW diluted with sterile distilled water (100%, 75%, 50%, and 25% v v^{-1}) as the culture medium. The assays were carried out in 6 L open raceway bioreactors (5 L working volume), with agitation provided by rotating paddles at 1.67 rad s^{-1} (16 rpm) and kept in a greenhouse with transparent film under outdoor conditions. The initial biomass concentration used was 0.2 g L^{-1}, and the assays lasted 15 d. Daily, the culture volumes were measured with freshwater to compensate for evaporation.[9]

Analytical Determinations and Kinetic Answers

Every 24 hours, parameters in the culture medium (pH, temperature and photon flux) and related to the *Spirulina* sp. LEB 18 growth (photosynthetic activity and biomass concentration - X_b) were evaluated. The X_b parameter was determined by measuring the optical density (670 nm) using a digital spectrophotometer (Shimadzu UV/VIS UVMini-1240, Kyoto, Japan), with a previously established standard curve.[9] The pH was measured directly with a digital pH meter (Mettler Toledo FiveGoTM, Schwarzenbach, Switzerland). The temperature was measured in the liquid medium using a digital thermometer (J.PROLAB SH 102, São Paulo, Brazil), and the photon flux ($\mu mol_{photons}$ m^{-2} s^{-1}) was measured using a universal light meter (WALZ ULM-50, Effeltrich, Germany). The efficiency of photosynthetic activity (Fv/Fm) was evaluated from the variable fluorescence (Fv) and maximum fluorescence

(Fm) of chlorophylls.[10] The maximum biomass concentration (X_{max}, g L^{-1}) and maximum productivity (P_{max}, mg L^{-1} d^{-1}) were evaluated. The volumetric productivity (P_X) was determined according to Equation 1, where X_t was the biomass concentration (mg L^{-1}) at time t (d) and X_0 was the biomass concentration (mg L^{-1}) at time t_0 (d).

$$P_x = \left(\frac{X_t - X_0}{t - t_0}\right) \quad (1)$$

For comparison purposes, the responses X_{max} and P_{max} were divided by the volume of freshwater (FW) used in each proposed condition and compared with the literature, resulting in the responses X_{max} V_{FW}^{-1} (g L^{-1} L_{FW}^{-1}) and P_{max} V_{FW}^{-1} (mg L^{-1} d^{-1} L_{FW}^{-1}), respectively. The average results of each treatment were statistically evaluated using analysis of variance (ANOVA) followed by Tukey's test, with a 95% confidence level.

3 RESULTS & DISCUSSION

The temperature of the cultures varied between 12.6 ± 1.9 °C and 38.3 ± 3.4 °C. Most microalgal strains exhibit optimal growth between 15 and 25 °C, but species of the *Spirulina* genus generally grow at higher temperatures, with optimal growth between 30 and 40 °C.[11] The variation in photon flux to which *Spirulina* sp. LEB 18 was subjected (108 ± 1 to 2221 ± 14 $\mu mol_{photons}$ m^{-2} s^{-1}) did not seem to hinder its growth (Figure 1a) and biomass production (Table 1). These results are similar to those found in studies with the same strain and similar experimental setup: 191.3 - 2300 $\mu mol_{photons}$ m^{-2} s^{-1} [9] and ~300 $\mu mol_{photons}$ m^{-2} s^{-1}. [12]

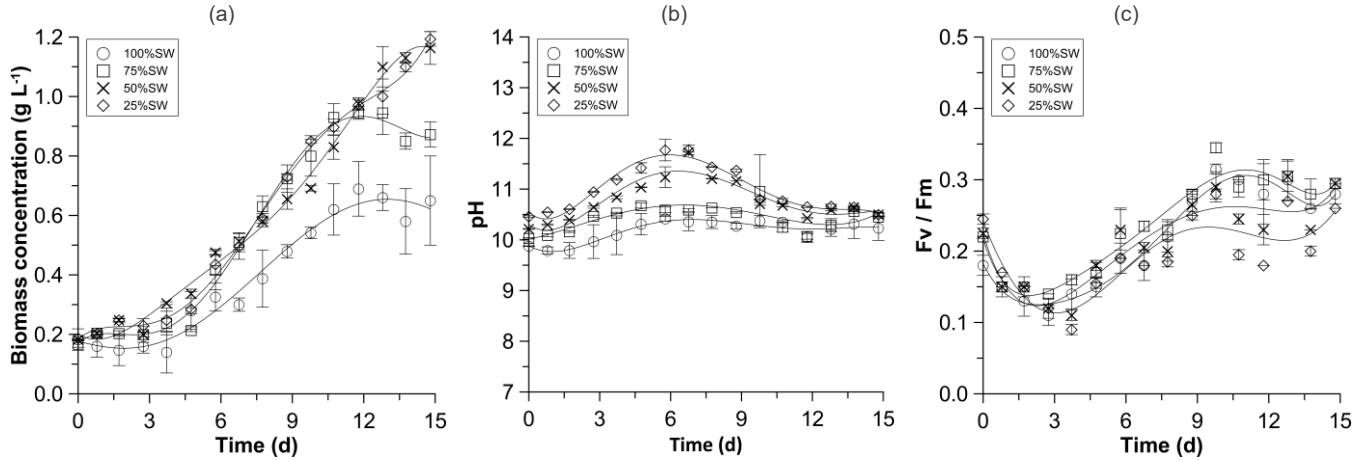

Figure 1 – Profiles of biomass concentration of *Spirulina* sp. LEB 18 (a), pH (b), and photosynthetic activity (Fv/Fm) (c) obtained in cultures with 100%, 75%, 50%, and 25% v v^{-1} seawater (SW).

Spirulina sp. LEB 18 growth profile with SW addition was similar until the 3rd day of the assay (Figure 1a). All evaluated conditions there was a phase of microalgae adaptation that lasted until approximately the 4th day of the experiment. From this day forwards, in the culture with the highest SW supply (100% v v^{-1}) there was no significant growth compared to the assays with 25% and 50% v v^{-1} of SW, resulting in the lowest biomass concentrations at the end of the batch. Thus, it was observed that concentrations of 50% and 25% v v^{-1} of AM sustained the growth of the strain until the end of the 15-day assay.

Table 1 - Results of maximum biomass concentration (X_{max}), maximum biomass productivity (P_{max}), X_{max} and P_{max} relative to the volume of freshwater used (FW) (X_{max} V_{FW}^{-1} and P_{max} V_{FW}^{-1}), obtained in assays with *Spirulina* sp. LEB 18 cultured with the addition of 100%, 75%, 50%, and 25% v v^{-1} seawater.

Parameter	Seawater concentration (v v^{-1})				Reference[9*]
	100	75	50	25	
$X_{máx}$ (g L^{-1})	0.68 ± 0.01^c	0.95 ± 0.01^b	1.20 ± 0.01^a	1.19 ± 0.08^a	3.16 ± 0.09
P_{max} (mg L^{-1} d^{-1})	42.4 ± 0.6^b	71.3 ± 5.0^a	75.9 ± 5.3^a	71.9 ± 0.8^a	260 ± 10
$X_{máx}$ V_{FW}^{-1} (g L^{-1} L_{FW}^{-1})	-	0.8 ± <0.01	0.5 ± <0.01	0.3 ± <0.01	0.6 ± <0.01
P_{max} V_{FW}^{-1} (mg L^{-1} d^{-1} L_{FW}^{-1})	-	57 ± 1	30 ± 1	19 ± < 0.2	52 ± 2

To treatments with seawater, equal superscript lowercase letters on the same line, indicate that the means do not differ statistically at the 95% confidence level ($p > 0.05$); *Control assay: 100% Zarrouk medium, outdoor environmental conditions, same strain and bioreactor as the present study.

The pH profile (Figure 1b) was similar throughout the assays. The pH range of maximum values was close to the 6th day. The assays with 25% and 50% v v^{-1} SW showed higher pH results. Increased photosynthetic activity in microalgae leads to higher concentrations of anions like hydroxide (OH^-) in the medium.[13] This corroborates the higher biomass concentration values (Figure 1a) since higher pH values are indicative of increased photosynthetic activity.

The Fv/Fm profiles (Figure 1c) confirm the adaptation phase of the cultures shown in Figure 1a, characterized by a reduction in photosynthetic efficiency until around the 4th day across all SW concentrations. After this period, there was an increase in the Fv/Fm results until the end of the batch. The average Fv/Fm result achieved was 0.3 for the highest AM concentrations and 0.2

for the lowest SW concentrations. The variation in Fv/Fm responses may indicate CO_2 fixation in the medium or photoinhibition, and its reduction is related to the decrease in the quantum yield of oxygen evolution or CO_2 fixation. [14] However, due to the outdoor cultivation conditions (uncontrolled light intensity and temperature) associated with higher salinity in the medium, the reduction in Fv/Fm responses can be explained.

The cultures with lower SW supplies (25% and 50% v v^{-1}) showed higher X_{max} results ($p < 0.05$) and were equal to each other ($p > 0.05$) compared to the other SW treatments (Table 1). The P_{max} values in the assays with 25%, 50%, and 75% v v^{-1} SW were higher ($p < 0.05$) than that observed in the assay with 100% v v^{-1} SW. These results indicate that the nutritional supply provided by SW may be sufficient for the growth and biomass productivity of the evaluated strain. However, the combination of outdoor conditions and the higher salinity tested (100% SW treatment) was detrimental to the growth kinetics of the strain.

The referenced literature[9] reports higher X_{max} and P_{max} values compared to all treatments using SW. Notably, these results[9] employed the same strain, bioreactor, and outdoor conditions, but with standard Zarrouk medium. However, when considering the freshwater used to obtain the literature results, which were 5 useful L per bioreactor, the results of X_{max} V_{FW}^{-1} (0.8 ± < 0.01 g L^{-1} L_{FW}^{-1}) and P_{max} V_{FW}^{-1} (57 ± 1 mg L^{-1} d^{-1} L_{FW}^{-1}) are higher when 75% v v^{-1} SW was used (Table 1).

Nutrients for large-scale *Spirulina* biomass production account for around 20% of total operating costs. Factoring in the need for freshwater, this cost can reach up to 35%, making it the second-largest operational expense.[3] Considering only the investment in nutrients from the Zarrouk cultivation medium, used for *Spirulina* strains, the approximate cost is US$0.20 g^{-1}.[15] Therefore, finding alternative sources of essential nutrients and reducing freshwater usage are crucial for large-scale *Spirulina* production. This would increase the economic viability and enhance interest in the segment and competitiveness of the process compared to other crops.

4 CONCLUSION

The lower SW concentrations (50% and 25% v v^{-1}) favored higher X_{max} results ($p < 0.05$), while P_{max} results with 25%, 50% and 75% v v^{-1} SW were superior ($p < 0.05$) the condition 100% v v^{-1} SW. The assay with 75% v v^{-1} SW showed kinetic results relative to using freshwater ~34% and ~10% higher for X_{max} and P_{max}, respectively, concerning the control culture (using 100% freshwater). Therefore, *Spirulina* biomass production with reduced freshwater use and under outdoor conditions appears feasible. Future studies could focus on achieving greater growth parameters by exploring alternative cultivation methods like semicontinuous mode or by supplementing specific nutrients like nitrogen and phosphorus sources.

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