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WINERY WASTEWATER and CO_2 BIOREMEDIATION THROUGH *Spirulina* CULTIVATION ADDED OF NANOFIBERS

Larissa C. Lopes[1], Gabriel M. Rosa[1]*, Michele G. Morais[2], Jorge A. V. Costa[1]

[1]*Laboratory of Biochemical Engineering,* [2]*Microbiology and Biochemistry Laboratory, College of Chemistry and Food Engineering, Federal University of Rio Grande, Rio Grande, Brazil.*
**Corresponding author's email address: gmdarosa@gmail.com*

ABSTRACT

Biorefineries generate high-value products from diverse raw materials with low environmental impact. Microalgae biomass is a promising, eco-friendly source for CO_2-neutral biofuels. Microalgae production offers reduced competition with food, minimal land and water use. However, industrial-scale production faces high cultivation costs and challenges. This study aimed to evaluate biomass productivity and CO_2 utilization efficiency from *Spirulina* sp. LEB 18 grown in winery wastewater with added nanofibers. The strain used was cultivated in effluent collected from a winery. Cultures were conducted using this effluent (25% and 50% v v^{-1}) in diluted Zarrouk medium without a carbon source, with nanofibers composed of polyacrylonitrile polymer and monoethanolamine. Results showed that the highest biomass concentrations and CO_2 biofixation rates were achieved with 50% v v^{-1} effluent + nanofibers. The effluent increased the photosynthetic efficiency of *Spirulina* sp. LEB 18 without adverse effects on growth. The assay conducted with 50% v v^{-1} effluent + nanofibers showed R_{max} and E_{max} 37% higher than the assay with 25% v v^{-1} + nanofibers. The results also demonstrated that winery effluent is a viable nutrient source for microalgae cultivation, supporting growth, CO_2 fixation and the production of biomass.

Keywords: Monoethanolamine. Microalgae. Nutrient removal. Biomass Productivity.

1 INTRODUCTION

Microalgae biomass is particularly promising for eco-friendly, CO_2 neutral biocompounds, such as carbohydrates, pigments, oils, and proteins, offering socio-economic and environmental benefits.[1] Microalgae offers advantages for bioproduct production compared to traditional crop cultivation: they require less land and water, boast shorter harvesting cycles, exhibit high biomass productivity, and demonstrate adaptability to diverse environments. Furthermore, the skill of microalgae for CO_2 biofixation is decisive in mitigating greenhouse gas emissions[1]. Nevertheless, large-scale microalgae production encounters challenges due to high costs of cultivation.[2] Optimizing cultivation media using wastewater or alternative resources can lower microalgae cultivation costs.[3]

Wastwater treatment is crucial for environmental preservation, especially in industries like wineries, where inadequate treatment can lead to water eutrophication. Wineries generate substantial waste, with 75% being effluents, significantly impacting the environment due to high organic load and production volume.[4] Current wastewater treatment methods have limitations, including high energy consumption and chemical use.[5] Using microalgae for wastewater treatment is a promising alternative, capable of removing nutrients like phosphorus and nitrogen, and integrating nanofibers into microalgae cultures can enhance CO_2 biofixation efficiency and nutrient removal, promoting sustainable solutions.[6,7] Overall, combining innovative technologies, such as microalgae and nanofibers, holds promise for sustainable biorefineries producing high-value-added bioproducts. In this context, this study evaluated the growth parameters and CO_2 biofixation potential of *Spirulina* cultivated in winery wastewater and added nanofibers.

2 MATERIAL & METHODS

Nanofibers composed of 10% (w v^{-1}) polyacrylonitrile polymer (PAN, MM 150.00 g/mol) and 1% (v v^{-1}) monoethanolamine (MEA, C_2H_7NO) were fabricated using electrospinning and added to the culture medium at 0.1 g L^{-1}.[7] The *Spirulina* sp. LEB 18 strain[8] (was sourced from the Culture Collection of the Biochemical Engineering Laboratory (LEB) at the Federal University of Rio Grande (FURG). Effluent was collected from a winery in Rio Grande do Sul, Brazil, after primary treatment. Cultures were conducted in diluted effluent (25% and 50% v v^{-1}) using modified Zarrouk medium[9] without a carbon source. both with and without nanofibers. Control experiments used Zarrouk medium with and without nanofibers. Experiments were conducted in duplicate within 2.0 L vertical tubular photobioreactors (1.8 L usable volume), with an initial biomass concentration of 0.2 g L^{-1}, 12-hour light/dark cycle with light intensity at 41.6 $\mu mol_{photons}$ m^{-2} s^{-1}, at 30 ºC, for 15 d. The stirring carried out with compressed air filtered through glass wool[10,11], and CO_2 was supplied for 1 min h^{-1} during the light period at 0.36 mL CO_2 mL_{medium}^{-1} d^{-1}.[12]

Biomass concentration was monitored daily using a spectrophotometer (Shimadzu UV/VIS UVmini-1240, Tokyo, Japan) at 670 nm, based on a standard curve relating optical density to dry biomass. Biomass concentration values were used to determine the maximum biomass concentration (X_{max}, mg L^{-1}) and maximum productivity (P_{max}, mg L^{-1} d^{-1}). Volumetric productivity (P_X) was calculated using the Equation 1, where x_t is the biomass concentration (mg L^{-1}) at time t (d) and x_0 at time t_0. The maximum CO_2 biofixation rate (R_{max}, mg L^{-1} d^{-1}) was calculated according to Equation 2,[13] using P_X values (mg L^{-1}), the molar masses of CO_2 (M_{CO2}, g mol^{-1}) and carbon (M_C, g mol^{-1}), considering the carbon fraction (X_{cbm}) in microalgal biomass as 47% (w w^{-1})[14]. Maximum CO_2 efficiency utilization (E_{max}, % w w^{-1}) was calculated using the Equation 3, where V (L) is the working volume of the

photobioreactors, and ṁ (mg d^{-1}) is the daily CO_2 feed mass rate.[15] Nitrogen[16] and phosphorus[17] concentrations were assessed every 72 h after sample filtration. Results were analyzed using ANOVA and Tukey's test with a 95% confidence interval.

$$P_X = \frac{x_t - x_0}{t - t_0} \quad (1)$$

$$R = P_x * X_{cbm} * \frac{MM_{CO_2}}{MM_C} \quad (2)$$

$$E = \frac{R * V}{\dot{m}} * 100 \quad (3)$$

3 RESULTS & DISCUSSION

Despite adverse conditions from the effluent, characterized by low pH and restricted nutrient supply, *Spirulina* sp. LEB 18 has demonstrated kinetics parameters highest ($p < 0.05$) to the control assay (Table 1). The highest biomass concentrations (X_{max}) and biomass productivity ($P_{máx}$) were achieved in assay with 50% v v^{-1} effluent + nanofibers, with no difference between the assay with 25% v v^{-1} effluent + nanofibers and the control condition ($p > 0.05$). X_{max} e P_{max} results, no statistically significant difference ($p > 0.05$) was observed between the control and the treatment containing the lowest effluent concentration combined with nanofibers. This suggests a potential nutrient limitation or insufficient removal in the assay with 25% v/v effluent + nanofiber, as indicated by Figure 1.

Table 1 Responses of biomass concentration (X_{max}), maximum biomass productivity (P_{max}), maximum CO_2 biofixation rate (R_{max}), and maximum CO_2 use efficiency (E_{max}), for *Spirulina* sp. LEB 18 cultivated in winery effluent, with added nanofibers.

Parameter	Control	50% v v^{-1} Effluent + Nanofibers	25% v v^{-1} Effluent + Nanofibers
X_{max} (g L^{-1})	2.58±0.39^b	3.14±0.48^a	2.51±0.11^b
P_{max} (mg L^{-1} d^{-1})	211.7±27.5^b	228.8±4.9^a	167.3±5.7^b
R_{max} (mg L^{-1} d^{-1})	-	394.3±8.4^a	288.3±9.8^b
E_{max} (% m m^{-1})	-	57.6±1.7^a	42.1±1.6^b

Equal lowercase letters on the same line for the same parameter indicate that the means do not differ statistically at the 95% confidence level ($p > 0.05$).

Regarding R_{max}, it can be observed that the assay with 50% v v^{-1} effluent + nanofibers have higher values ($p < 0.05$) than the assay with 25% v v^{-1} effluent + nanofibers. The R_{max} result from the lower effluent test was 27% lower than the answer obtained from the higher effluent assay. The reduction in R_{max} observed in the group with 25% v v^{-1} effluent + nanofibers may be related to the composition of the assay or the nutritional availability of the medium culture[18]. Additionally, the concentrations of available nutrients in the medium may play an important role in the CO_2 biofixation. The results with 50% v v^{-1} effluent + nanofibers indicated that this condition favored a R_{max} 37% higher than the other assay.

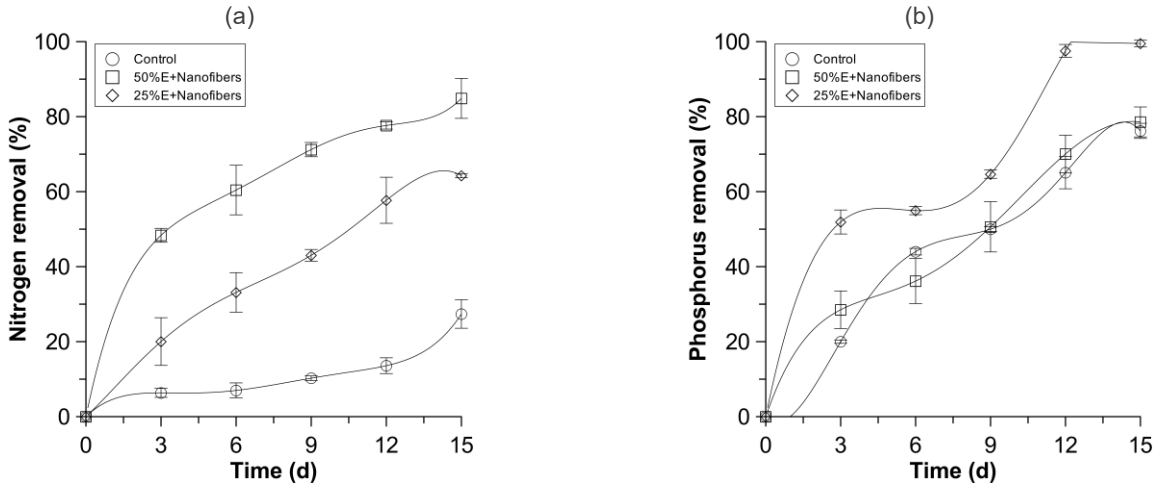

Figure 1 Nitrogen (a) and phosphorus (b) removal profiles in the cultivation of *Spirulina* sp. LEB 18 in in winery effluent (E) + nanofibers diluted modified Zarrouk medium (without carbon source).

The CO_2 utilization efficiency (E) by microalgae can be influenced by factors such as effluent composition, the presence of essential nutrients, and light availability[18]. The assay with 50% v v^{-1} winery effluent + nanofibers showed significantly highest maximum CO_2 utilization efficiency (E_{max}) ($p < 0.05$) compared to the 25% v v^{-1} effluent + nanofibers. These results suggest that the treatments used are effective in promoting CO_2 utilization efficiency by *Spirulina* sp. LEB 18. In this context, the nutrients

present in the effluent used may play a crucial role in the response by the microalgae. Additionally, the availability of essential nutrients, such as nitrogen and phosphorus, is decisive for the growth and metabolism of the microalgae.

As previously attested, the addition of nanofibers to the culture medium could improve E results by the microalgae, providing an additional surface for microalgae adhesion and growth, thereby increasing the CO_2 fixation rate[7]. However, several factors may interfere with the interaction between the nanofibers and the microalgae, including pH variations in the effluent, contact time in the medium, compatibility between the nanofibers and the strain, and the concentration of nanofibers used.[7,18]

Across all experimental conditions, nitrogen concentrations showed a significant reduction throughout the study period, indicating efficient nitrogen uptake by *Spirulina* sp. LEB 18 (Figure 1a). Maximum removal rates ranged from 64% to 85%, with the highest observed in assays containing 50% v v^{-1} effluent + nanofibers. This efficient nitrogen utilization highlights the potential of strain for bioremediation of nutrient-rich winery wastewater. *Spirulina* sp. LEB 18 demonstrated high efficiency in phosphorus removal (Figure 1b), achieving removal rates between 79% and 99%. The highest removal was observed in experiments with a 25% v v^{-1} effluent + with nanofibers. These removal rates suggest that the strain effectively utilized the available phosphorus in the effluent, contributing to its growth and productivity.

The results demonstrated that the strain effectively utilized the nutrients present in the effluent, particularly nitrogen and phosphorus, which are essential for its growth and metabolic activities. These results suggest that winery effluent can serve as a viable nutrient source for microalgae cultivation, effectively supporting the growth and biomolecule production of *Spirulina* sp. LEB 18.

4 CONCLUSION

The results showed that the assays with winery effluent and nanofibers significantly increased ($p < 0.05$) the biomass concentration and productivity of *Spirulina* sp. LEB 18 compared to the control assay. The assay conducted with 50% v v^{-1} effluent showed R_{max} and E_{max} values that were 37% higher ($p < 0.05\%$) than those in the assay with 25% v v^{-1} effluent. The nutrient consumption results indicate that the strain efficiently utilized nitrogen and phosphorus from the effluent, demonstrating that winery effluent serves as a viable nutrient source for growth and biomolecule production. Therefore, combining nanofibers with winery effluent could enhance the sustainability and efficiency of microalgae cultivation, thereby promoting greater CO_2 biofixation.

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