PILOT SCALE ON-SITE BIOREMEDIATION OF BREWERY WASTEWATER USING *Arthrospira sp.* IN RIO DE JANEIRO, BRAZIL: A PRELIMINARY ASSESSMENT OF BIOMASS AS A BIOFERTILIZER FOR BARLEY (*Hordeum vulgare*)

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ABSTRACT

Microalgae have been pointed out as promising agents for bioremediation of wastewater from numerous sources. One of the main advantages of such technology is the production of valuable biomass to be exploited in other processes, increasing the sustainability and circularity of the system. In this study, untreated brewery wastewater was used as culture medium for *Arthrospira* sp. on a pilot scale setup positioned within the production site of Rio de Janeiro brewery(Rio de Janeiro, Brazil). The resulting biomass was then preliminarily assessed as a biofertilizer for barley (*Hordeum vulgare*) growth. After 100 days of semi-continuous cultivation with a weekly dilution rate of 0.35, the resulting dry biomass was finally tested as a biofertilizer in different degrees of substitution of a commercial mineral fertilizer. Results showed that, using optimal inoculum dilution of 50%, the onsite pilot scale cultivation of *Arthrospira* sp. using Nova Rio's brewery effluent reached an average biomass productivity of 10.68 ± 5.13 g m^{-2} d^{-1}. Nitrate, ammonium, phosphate, sulfate, fluoride, and organic carbon were successfully removed according to the Brazilian regulations. Preliminary biofertilization assays showed that 50 and 100% substitution of commercial fertilizer for *Arthrospira* sp. biomass resulted in faster barley growth, indicating a promising venture to be further studied.

Keywords Microalgae, *Arthrospira* sp., Brewery wastewater, Biofertilizer, *Hordeum vulgare*

1 INTRODUCTION

Microalgae are often pointed out as promising agents for bioremediation of effluents from diverse sources. Employing these microorganisms in such treatment poses several advantages as they can remove a wide array of contaminants, fix atmospheric CO_2, and yield valuable biomass during the process (PACHECO et al, 2020). Moreover, macro- and micro-algae – whole cells or extracts – have been consistently reported in the literature as promising biostimulants and biofertilizers for many crops of commercial interest (WIN et al, 2018 doi 10.1089/ind.2018.0010; CHATTERJEE et al, 2017 doi 10.1016/B978-0-444-63784-0.00010-2). Among the huge diversity of microalgae, cyanobacteria belonging to the genus *Arthrospira* (commonly called Spirulina) stand out as the most studied, produced, and commercially exploited microalgae worldwide (LAFARGA et al, 2021).

In this study, *Arthrospira* sp. was cultivated in brewery wastewater from Nova Rio brewery (Rio de Janeiro, Brazil) as a bioremediation agent, and the resulting biomass was tested as a biofertilizer for barley (*Hordeum vulgare*), one of the main beer ingredients. This way, microalgae biotechnology was used to simultaneously mitigate two issues confronted by the brewing industry, increasing sustainability and circularity. First, the optimal dilution for inoculum was determined in lab scale cultures that were then scaled up to start a pilot scale cultivation of *Arthrospira* sp.. After 100 days of pilot scale cultivation, the resulting dry biomass was finally tested as a biofertilizer in different degrees of substitution of a commercial mineral fertilizer. The results encourage further larger scale studies on the use of microalgae to solve environmental issues of the brewing industry, while increasing its sustainability and circularity.

2 MATERIAL & METHODS

Organism and culture conditions: *Arthrospira* sp. was kindly provided by the Microalgae Collection from the Laboratory of Studies Applied to Photosynthesis (CMLEAF), in the Chemistry Institute of the Federal University of Rio de Janeiro, Brazil. Cultures used as inoculums were maintained at 30 ± 2°C exposed to

120 µmol photons m^{-2} s^{-1} illumination with 12:12 h light : dark cycles and constant orbital agitation of 156 rpm. Growth medium (pH 9.4) contained per liter: 13.61 g $NaHCO_3$, 4.03 g Na_2CO_3, 0.50 g K_2HPO_4, 2.50 g $NaNO_3$, 1.00 g K_2SO_4, 1.00 g NaCl, 0.20 g $MgSO_4$ $7H_2O$, 0.04 g $CaCl_2$ $2H_2O$, 0.01 g $FeSO_4$ $7H_2O$, 0.08 g Na_2EDTA $2H_2O$, 1.00 mL vitamins solution, 1.00 mL trace metals solution (AO medium; Aiba and Ogawa, 1977). Cells were sub-cultured on a weekly basis in order to be maintained in exponential growth phase during the whole set of experiments.

Effluent characterization: The brewery effluent was collected from the mechanized grid of the Nova Rio brewery's industrial effluent treatment plant (Rio de Janeiro, Brazil), and no effluent pretreatment was carried out before using it for microalgae cultivation. When necessary – i.e., lab-scale trials –, it was stocked at room temperature, sheltered from direct light for no longer than 24 hours. Physicochemical characterization of the effluent used in this study was carried out..

Lab scale determination of culture conditions: Optimal dilutions for inoculums were tested in 500 mL Erlenmeyer flasks containing 250 mL of different proportion mixtures of microalgal culture and brewery effluent in the same conditions as described in Section 2.1 for 14 days. The tested dilutions were 20 % (50 mL inoculum), 30% (75 mL inoculum), 40% (100 mL inoculum), and 50% (125 mL inoculum) carried out in three biologically independent replicates each. Microalgal growth was followed daily by taking samples for optical density (750 nm and 682 nm) and dry weight determination. Considering the intrinsic microbiota and variable turbidity of the effluent, microalgal growth was followed spectrophotometrically by the ratio between absorbance at 682 nm (peak absorbance of chlorophyll) and at 750 nm (light scattering). Specific growth rate (µ) was estimated from the linear coefficient of the equation describing the exponential portion of the logarithmic growth curve.

On site pilot scale trials: After scaling up to 150 L microalgal culture on tubular photobioreactors using the best inoculum and dilution rate resulting from experiments described in Section 2.3, a 1.55 m^2 glass fiber open raceway pond (nominal capacity of 1,000 L) was inoculated to an initial working volume of 300 L. The pond was located at the Nova Rio Technology and Innovation Center (CIT), Rio de Janeiro, Brazil (22°51'S, 43°13'W), exposed to environmental conditions and weathering, and equipped with a paddlewheel that agitated the algal culture with a surface water speed of 0.14 - 0.16 m s^{-1}. The final working volume was, thus, dependent on environmental water dynamics by evaporation and pluviosity. Main physicochemical parameters of light intensity, culture temperature, salinity (S-10 refractometer, Shibuya, Japan), and pH (pH meter 913, Metrohm, Switzerland) were daily monitored. Light intensity was measured on the surface of the algal culture using a LI-250A radiometer (Li-Cor, Germany), and pluviosity data was retrieved from the Brazilian National Meteorological Institute (INMET).

3 RESULTS & DISCUSSION

3.1 Lab scale determination of microalgal inoculum on brewery effluent

All initial dilutions for inoculums were able to sustain microalgal growth at a certain level, indicating that the untreated effluent had no toxic effects on *Arthrospira* sp. In all treatments, cyanobacterium cells presented a lag phase, represented by a decrease on the optical density ratio (682 nm : 750 nm) during the first three to five days of cultivation, before exponential growth started. Specific growth rates (µ) ranged from 0.088 to 0.149 d^{-1} in 30 and 50% inoculum dilution treatments, respectively. The lowest growth rate values, belonging to 20 and 30% dilution treatments, were not significantly different ($p > 0.05$). After around five days of exponential growth, cell division rate slowly decreased until stationary phase was reached, and cell yield remained constant. Maximum dry biomass yield ranged from 1.67 to 3.20 mg mL^{-1} for 20 and 40% dilution treatments respectively. Final yields from 30, 40, and 50% dilution treatments, however, were not significantly different ($p > 0.05$).

3.2 Pilot scale onsite cultivation of *Arthrospira* sp.

Based on the results presented in Section 3.1, upscaling operation was performed using 50% dilution for inoculum, which resulted in faster growth and higher dry biomass yield. Results from the 100-days (March to June) onsite cultivation of *Arthrospira* sp. in 1.55 m^2 open raceway pond were reported daily. Maximum daily light intensity was around 1,500 µmol photons m^{-2} s^{-1} with a wide variation of values, whereas temperature ranged from 17 to 29 °C with a clear decreasing trend during the experimental period. Likewise, pH values generally decreased during cultivation of *Arthrospira* sp., ranging from 8.8 to 9.5. Salinity values tended to increase from 0.6 to 5.5 ‰ during the experiment, reasonably contrasting to pluviosity, which reached a maximum of 78.4 mm at the third week, and then consistently decreased as expected for the period in question.

3.3 Preliminary analysis of *Arthrospira* sp. biomass as biofertilizer

After 40 days in a controlled environment, the aerial portion of *H. vulgare* plants had, on average, 56.3 ± 1.7 cm with no statistically significant difference among them. Likewise, at the end of the experiment, the length of the plants was essentially the same, averaging 74.2 ± 2.3 cm. On the other hand, the growth rate during the 60-days period of daily monitoring had different behavior among the treatments. Whereas the control (0%) and the 20% substitution treatment were not statistically different throughout the whole experiment, the 50% treatment showed faster initial growth when compared to the others, being significantly higher from day 45 ($p < 0.05$). After that, from day 54, the 100% substitution treatment reaches similar values to the 50% treatment, as both became significantly higher than the other two treatments ($p < 0.05$). By days 55 – 60, the 50 and 100% substitution treatments had reached values corresponding to their final length, which happened to the other treatments only around days 75 – 79.

4 Discussion

4.1 Optimal brewery effluent inoculum on untreated brewery effluent

Raw brewery wastewater usually presents high levels of particulate material, turbidity, and autochthonous consortium of microorganisms that may inhibit the growth of bioremediation agents (AMENORFENYO et al, 2019). Such characteristics, among others, indicate that a pretreatment can be desirable before the introduction of microalgae into the system. A wide array of pretreatment methods has been reported, such as filtration, autoclaving, chlorination, and ultraviolet radiation (PAPADOPOULOS et al, 2022; RAMSUNDAR et al, 2017; QIN et al, 2014). However, the application of such pretreatment methods in large scale setups may not be energetically and/or economically feasible (ACIEN et al 2019). Based on this perspective, promising results regarding the use of microalgae for bioremediation of brewery wastewater with absent or minimal pretreatment have been reported.

4.2 Pilot scale trials - The constraints of scaling up

Today, the literature on applied phycology is vast, extremely informative, and provides great amount of evidence that microalgae would be the ideal bioagents for increasing sustainability and circularity of several traditional industrial processes (KUMAR et al, 2024; MOREIRA et al, 2023). However, real exploitation of microalgae in industries has been incipient so far, with few exceptions mainly in the food sector. Pilot- to commercial-scale trials usually result in lower biomass output rates and economic feasibility when compared to lab scale results found in the literature, discouraging new ventures (GROBBELLAR, 2012). This scenario highlights the importance of studies that, like this one, transcend the walls of a laboratory and its volumetric limitations. The results reported here describe a feasible low-cost industrial protocol that maintained a productivity of 10.68 g m^{-2} d^{-1} (~0.06 g L^{-1} d^{-1}) for 100 days and efficiently removed major contaminants. Likewise, using *Arthrospira platensis* (SAG 21.99) in 1 L batch cultures, PAPADOPOULOS et al (2022) reached a productivity of 0.07 g L^{-1} d^{-1} and successfully removed nitrate and phosphate from untreated brewery wastewater. Interestingly, it has been also shown that *A. platensis* was not able to thrive in brewery effluent from other source (RAPOSO et al, 2012).

4.3 Preliminary biofertilization assays and future perspectives

Microalgal biomass from bioremediation can increase sustainability and circularity of a production system when employed in upstream processes, provided that it is permitted by local environmental regulations (SUTHERLAND and RALPH, 2019). In this case study, for instance, *Arthrospira* sp. biomass grown in brewery wastewater was tested as a biofertilizer for barley, a brewing ingredient. Mostly using *Arthrospira* extracts, other studies have shown positive effects on seed germination of barley and wheat; growth and photosynthetic capacity of yellow lupin (*Lupinus luteus*), and root development of watercress (*Lepidium sativum*) (AKGUL, 2019; SHEDEED et al, 2022; VILLARO et al, 2023). However, to the best of these authors knowledge, no evaluation of biofertilization effects of whole *Arthrospira* cells on barley was carried out so far. It has been consistently reported in the literature that the nitrogen concentration of *Arthrospira* spp. whole cells mildly varies around 10% (ALMEIDA et al, 2017; CASAZZA et al, 2020; MAHROUQI et al, 2022).

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