BIOMASS PRODUCTION BY *Chlorella* spp. ISOLATED FROM EFFLUENT TREATMENT STATION OF PARBOILED RICE INDUSTRY

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1. INTRODUCTION

Rice is one of the most important and widely consumed cereal worldwide. Food and Agriculture Organization (FAO) and United States Department of Agriculture (USDA) forecast a world rice production between 487.8 and 513 million tons for 2018/19; Brazil production is estimated to raise 136,000 tons to 8.16 million tons for the same period based on a 20-year trend updated in 2017/18 by Government of Brazil report (USDA, 2018) and is projected to be the largest exporter of rice in South America. Parboiled rice is one of the many products obtained from rice productive chain and nearly 25% of Brazilian rice is designated to this sector (PARAGINSKY et al., 2014).

The parboilization process use rice with hull, where the grain with all its components is soaked, steamed and dried, then passing by hull removal. The hydrothermal process enables the rice to absorb nutrients and produce a regular and firm grain due to changes in starch. The parboilization demands a high volume of water and generates an average volume of 2 L of parboiled effluent (PE) per kg of rice (GABOARDI et al., 2018). In addition to the volume, PE is recognized by environment risk due the high levels of nutrients and the unavailability of adequate treatment techniques, demanding the development of new approaches with PE. Biological techniques are a trend due to possibility of nutrient reuse, costs reduction, environmental protection and sustainability – aspects that are harder to obtain applying physicochemical methods on the treatment station.

Microalgae are photosynthetic microorganisms useful on treatment stations due to rapidly grow, ability in incorporate difficult-removal nutrients such as phosphorus and nitrogen, widely application of generated microalgae biomass, CO_2 fixation and energy recovery (BRENAN & OWENDE, 2010). Residues as agricultural wastes (PHANG & ONG, 1988), toxic minerals (XIAOHUA et al., 1995) and also on parboiled effluent (MUKHERJEE et al., 2016) has been treated with different microalgaes.

The isolation of wild microorganisms from effluent treatment stations or contaminated sites is interesting due to adaptation to nutrient levels and environment, increasing the bioremediation potential. Besides that, exogenic microorganisms generally fail or shows low activity on bioremediation process since the environment is different from controlled conditions. The tertiary treatment station of parboiled rice industry represents an adequate niche for microalgae development due to nutrient concentrations and exposure to sun. The objective of this study was isolate a promising microalgae from effluent treatment station and evaluate the biomass production in parboiled rice effluent.

2. METHODOLOGY

2.1 Effluent collection and storage

PE was obtained from a local industry at the city of Pelotas – Brazil (latitude 31.646790, longitude 52.340367). Samples were collected from the parboilization process in sterilized plastic vials. The PE samples were sterilized by autoclaved for 25 min and stored at 4 °C until used.

2.2 Microalgae isolation and experimental conditions

Samples from the last pound of rice effluent treatment station where collected in sterile vials of 50 mL and immediately transported at room temperature to Microbiology Laboratory – UFPEL (Brazil), stored at 2 °C. BG11 agar plates (Himedia®) were prepared to isolate microalgae and cyanobacteria from treatment station: 60 µL of collected sample were transferred to each of a triplicate BG11 agar plate and incubated for 7 days, 28 °C, light/dark cycle (16:8) and 2000 lux. Colonies with different morphology were then selected and transferred to different Erlenmeyer flasks containing 200 mL of Watanabe, consisting of 1.25 g L^{-1} KNO_3 (NEON), 1.25 g L^{-1} KH_2PO_4 (Synth), 20 mg L^{-1} $MgSO_4$ (Synth), 20 mg L^{-1} $FeSO_4$ (Synth) and 1 ml L^{-1} of A5 solution (2.9 g L^{-1} H_3B_3O, 1.81 g L^{-1} $MnCl_2$, 0.08 g L^{-1} $CuSO_4$, 0.018 g L^{-1} $(NH_4)_3O_7MoO_3$ and (Synth) 0.11 g L^{-1} $ZnCl_2$), cultivated for 7 days at 28 ºC, light/dark cycle (16:8) of 2000 lux and pH 7. The mediums were centrifuged at 2000 g (Kubota - KR 600) for 10 min and the pellet inoculated in Erlenmeyer flasks with 200 mL of PE with pH 7 and cultivated for 7 days, 28 °C, light/dark cycle (16:8) and 2000 lux.

The microalgae with higher cell number in Neubauer chamber was selected and culture centrifuged at 2000 g (Kubota - KR 600) for 10 min. The pellet of biomass was weighty on analytical balance (Shimadzu - ATY224) and inoculum prepared in PE with 0.5 g L^{-1} of biomass and 20% v/v. The experiments were carried out in 4 L column photo-reactor with 3 L of PE and culture for 18 days, 28 °C, light/dark cycle (16:8), pH 7, 2000 lux and aerated. The regime of operation was maintained fed-batch since the volume lost with evaporation was replaced. Every 48 h the pH was measured and calibrated to 7. A culture in Watanabe medium was realized for grow and biomass control at same experimental conditions as PE.

2.3 Biomass

Biomass quantification (g) was made collecting a triplicate sample of 10 mL from each media at intervals of 48 h and transferring to sterile vials. The samples were centrifuged at 2000 g (Kubota - KR 600) for 10 min and pellet washed two times with sterile water before let dry at 60 ºC until constant weight. The dried pellets were weighted in analytical balance (Shimadzu - ATY224).

2.4 Statistical analysis

The results were analyzed on *Statistica* software version 10 (Statsoft) by *student's t-test* comparing means and $p<0.05$ was considered significant. All experiments were done, at least three times in triplicates.

3. RESULTS AND DISCUSSION

A wide variety of cells were first obtained in BG 11 plates from tertiary effluent treatment station. Nonetheless, isolated cells were under lower

concentrations of nitrogen, phosphorus and organic matter than in parboiled effluent (PE). To select resistant and viable strains, a culture in PE at pH 7 was realized, obtaining 2 strains. This culture in PE also could lead to higher growth as reported by Mukherjee et al. (2016) that obtained better results with pre-acclimatized microalgae and cyanobacteria. The isolate was selected quantitatively and morphological compared to algae bank (GUIRY, 2019), identified as *Chlorella* spp. The objective of this research was obtaining a strain able to grow and produce biomass in PE medium, and the identification of obtained strain was not focused.

Chlorella species are primary producers in different ecosystems, easily adapted, versatile in mass cultivation systems and widely applicated biotechnologically (KRIENITZ & BOCK, 2012). The *Chlorella* biomass is a product of economic interest and a low production cost is essential to make the process viable. Waste-grown microalgae shows as potential solution for remediation of high nutrient levels in industrial effluent and non-expensive system to produce biomass, when compared to standard mediums. *Chlorella* spp. maximum biomass is generally between 0.3 and 0.5 g L^{-1} when cultivated in standard medium as BG11 (LI et al, 2010). The biomass obtained in parboiled effluent (PE) is presented on Figure 1 and reached a maximum of 3.15 g L^{-1} at 18 d in a fed-batch culture and was higher than observed by many authors.

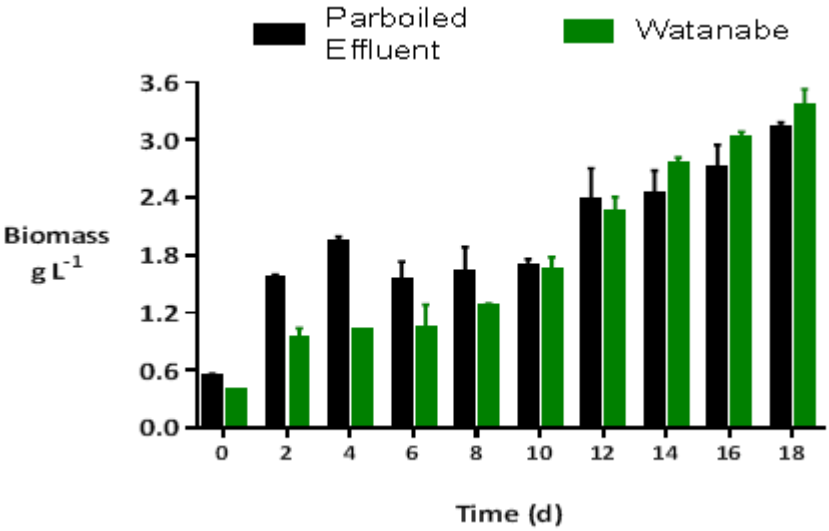

Figure 1: Biomass production in parboiled effluent and Watanabe medium. The data represents the biomass (mean +/- SD) at 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 d in parboiled effluent and Watanabe inoculated with *Chlorella* spp.

The rice industry generates diary an average volume of effluent of 650 m^3. The conversion by the maximum biomass yield obtained could produce 2.047 kg of *Chlorella* spp. per day. However, more research is needed to upscale, improve the microalgae multiplication and also direct the *Chlorella* spp. metabolism to accumulate specific substrates of commercial interest. Mature cells of *Chlorella vulgaris* can accumulate a total protein content between 42-58% (SERVAITES et al., 2012), 5-40% of lipids, 12-55% of total carbohydrates in low concentrations of nitrogen (CHOIX et al., 2012). Many other products can be extracted from *Chlorella* biomass as pigments (chlorophyll, carotenoids, astaxanthin, cantaxanthin), minerals (potassium, magnesium, zinc) and vitamins (B1, B2, B3,

B5, B6, B7, B9, B12, E and A) (PANAHI et al, 2012). Thus, *Chlorella* spp. biomass can be a co-product from rice productive chain due to wide application and industrial interest.

4. CONCLUSIONS

We successfully isolated two strains from the tertiary treatment station of parboiled rice effluent and evaluated the biomass of chosen microalgae due to cell number and over reproduction, morphologically identified as *Chlorella* spp. The biomass yield reached a high level and presents as coproduct. More studies are necessary to analyse biotic and physical factors, nutrient uptake and grow parameters to improve biomass yield.

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