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Nutrient Removal and Biomass Production by Culturing *Saccharomyces Cerevisiae* in Parboiled Rice Effluent

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ABSTRACT

The objective of this study was to evaluate the potential of *Saccharomyces cerevisiae* in the treatment of rice parboiling effluent (PE) and biomass production. PE was inoculated with *S. cerevisiae* at 1.2×10^4 CFU mL^{-1} and cultured in shaker at 28 °C and 180 rpm for 72 h. PE supplied the required nutrients for *S. cerevisiae* growth, reaching a biomass of ± 8.2 $g\cdot L^{-1}$, cell viability of ± 2×10^{11} CFU mL^{-1} and removals of 74%, 56% and 17% for total Kjedahl nitrogen, chemical oxygen demand and phosphorus, respectively. The versatile of *S. cerevisiae* supported the direct and non-supplemented cultivation in PE, resulting in high removals of nutrient and biomass production and represent an alternative method to reduce the environmental impact of rice industry and an alternative process to obtain marketable yeast biomass.

Keywords: *Saccharomyces cerevisiae*; parboiled effluent; bioremediation; biomass; cell viability

INTRODUCTION

Parboiled rice is one of main products from rice productive chain and is obtained by partially boiling the raw rice (Abinandan et al. 2015). This hydrothermal process occurs before milling and requires high volumes of potable water to partially cook the cereal, generating a volume of effluent that can reach 2 L per kg of rice processed (Abinandan et al. 2015; Gil de los Santos et al. 2012). Nowadays, the effluent from parboiling process is treated through systems that integrate the use of anaerobic reactors and activated sludge. However, a supplementary treatment to reach the discharge limits established by environmental agencies is necessary due to the inefficiency in removing nutrients as phosphorus and nitrogen (Kumar et al. 2017). The parboiled effluent (PE) lacks toxic compounds, nonetheless, contains high concentrations of nitrogen, phosphorus and organic matter. Traces of pesticides have been also detected in PE and is associated to local culturing methods (Amato et al. 2002; Karunaratne, 2002).

The biological treatment of industrial effluents represents an eco-friendly and highly efficient alternative when compared to classical physicochemical methods – microorganisms can easily adapt to adverse conditions of industrial effluents and metabolize the nutrients through a complex set of biochemical pathways. Several biological approaches such as aquatic plants, algae and yeast culture have been evaluated for the treatment of PE (Bastos et al. 2015; Fehrenbach et al. 2021; Gaboardi et al. 2018; Gerber 2002; Gil de los Santos et al. 2012; Mukherjee et al. 2016). The reported methods present different levels of nutrient

removal, cost and complexity. The socioeconomical importance of parboiled rice and the urgent demand for new treatment technologies to maintain sustainability and competitivity of this sector is highlighted by the rice production level. In 2020/2021, the global production of rice reached 507 million of metric tons – Brazil is one of the main producers and was responsible for 10.6 million of metric tons for the same period (Shahbandeh, 2020).

Saccharomyces cerevisiae is one of the most important and versatile yeast strains, its widely employed in the fermentation process at beverage and food industries. The bioremediation potential of *S. cerevisiae* has been already reported for parboiled effluent pre-treated with enzymes (Fehrenbach et al. 2021), vinasse (Rodrigues Reis and Hu, 2017), landfill leachate (Wichitsathian et al. 2004) and effluents with heavy metals (Machado et al. 2010). *S. cerevisiae* is certified as Generally Recognized as Safe (GRAS), and if cultured in a non-toxic media its biomass can be used as source of single cell protein (Lapeña et al. 2020), applied in the animal nutrition, probiotic drug, and as general as source of nutrients (Ferreira et al. 2010). Therefore, the objective of this study was to investigate the bioremediation potential and biomass production of culturing *Saccharomyces cerevisiae* in parboiled rice effluent.

MATERIAL AND METHODS

Sampling of parboiled effluent

Parboiled effluent (PE) was obtained from a local industry in the city of Pelotas–Brazil (31º 46' 19" S, 52º 20' 33" W). Samples were collected directly from the maceration process every 4 months for 1 year. The average levels (mg L^{-1}) of total solids (TS), chemical oxygen demand (COD), Total Kjeldahl nitrogen (TKN), total phosphorus (P) in this period were 717.5 ± 28.61, 568.37 ± 22.63, 142.25 ± 9.47, 79.45 ± 9, respectively, and pH 4.1 ± 0.25. The pH in PE was adjusted to 5.5 with a 1M NaOH solution and sterilized for 15 min at 121 °C.

Yeast and experimental methodology

Saccharomyces cerevisiae strain YT001–YEASTECH was kindly supplied by the Biotechnology Center of the Federal University of Pelotas (UFPel), Brazil. The inoculum was prepared transferring a colony of *S. cerevisiae* to yeast malt broth (YM) and cultivated at 180 rpm in shaker (Certomat BS-TA) and 28 °C for 24 h. Then, the inoculum was centrifuged at 2500 × *g* for 10 min and the pellet washed twice with distilled water to remove residual nutrients of YM. The cultivation was realized in non-aerated baffled flasks of 1 L, containing 200 mL of parboiled effluent (PE) and YM media (control media), inoculated with 10% (v/v) of *S. cerevisiae* at 1.2×10^4 CFU mL^{-1} and agitated at 180 rpm for 72 h. The pH in PE and YM was adjusted to 5.5 as *S. cerevisiae* is an acidophilic organism. In non-published results, the highest cell viability, biomass and removals were identified in pH 5.5. Yeast growth results and biomass were analysed in PE and YM, while chemical oxygen demand, total nitrogen and phosphorus removals in PE.

S. cerevisiae growth and biomass determination

The growth of *S. cerevisiae* was evaluated by counting the colony forming units (CFU mL^{-1}), collecting three replicates of 500 µL from PE and YM at 0, 12, 24, 36, 48 and 72 h of culture. The analysis was extended to 72 h in order to verify the presence of a decline phase. Samples were serially diluted with distilled water, 20 µL of each dilution spread onto YM agar plates and incubated at 28 °C for 48 h. The number of colonies was counted and CFU mL^{-1} obtained by converting to mL (*50) and corresponding dilution factor.

$$CFU\ mL^{-1} = average\ colonies\ counted \times 50 \times dilution\ factor \quad (1)$$

To quantify the *S. cerevisiae* biomass (g), samples of 10 mL were collected at 0, 12, 24, 36, 48 and 72 h from PE and YM culture and centrifuged at 2500 *g* (Kubota – KR 600) for 15 min. The pellet was washed twice with sterile water before dried at 60 °C and weighted in analytical balance (Shimadzu – ATY224) until a constant weight was obtained indicating the absence of humidity.

Analytical determination of total nitrogen, chemical oxygen demand and phosphorus

Triplicate samples of 500 µL from PE were collected for total nitrogen (TKN), phosphorus (P), and chemical oxygen demand (COD) determination at 0, 24 and 48 hours. The determination of sampling time point was based in the industry necessity since longer treatments than 48 h are considered inviable and require several modifications at the current treatment systems, projected for shorter hydraulic retention time.

Samples were stored frozen until analysis and thawed at room temperature (± 25 °C), centrifuged (Kubota – KR 600) at 3000 *g* for 5 min and the supernatant was collected for analysis. Total nitrogen was determined by HI93767B-50 Hanna TKN kit, transferring 500 µL of sample supernatant to a digestion tube containing potassium persulfate. A heated block was used to heat the digestion tube up to 105 °C for 30 min. At room temperature, sodium metabisulfite was added and gently agitated. After 3 min of reaction, total nitrogen reagent was added and left reacting for 2 min. From this solution, 2 mL were transferred to colorimetric reagent vial and analysed in photometer Hanna HI8339902. The absorbance was internally converted by the photometer to TKN. The same was observed for COD.

COD was determined by HI93754C-25 Hanna COD kit. A volume of 200 µL of supernatant was transferred to digestion tubes containing dichromate and the digestion was realized at 150 °C for 2 h. The colorimetric reaction was analysed in photometer Hanna HI8339902.

Phosphorus was determined by colorimetric method of phosphate vanadium molybdate (NBR 12772, 1992). A phosphate (PO_4^{3-}) standard curve at concentrations of 1, 2.5, 5, 7.5 and 10 mg L^{-1} was prepared using a 1000 mg L^{-1} stock. The concentration of phosphate was estimated replacing the *y* for the absorbance in the follow equation:

$$y = 0.0303x + 0.0045 \; R^2 = 0.9998 \qquad (2)$$

Samples were analysed transferring 2 mL of molibdate-antimonium (LabSynth) to 10 mL volumetric flask followed by respective sample and dilution. The determinations were made in triplicate and verified on UV-VIS (Kasuaki – IL592) at 400 nm, quantifying the colorimetric intensity of the complex antimonium-phosphate-molybdate. Phosphorus (mg L^{-1}) was obtained converting the phosphate results through molecular weight.

Statistical analysis

The results were analysed on software *Statistica* version 10 (Statsoft, TIBCO Software Inc., Palo Alto, CA 94304, USA) by Student's t-test comparing means and $p<0.05$ was considered significant. All experiments were done, at least three times in triplicates.

RESULTS

Saccharomyces cerevisiae growth and biomass

S. cerevisiae growth was studied until 72 h of culture. In the first two hours of culture, no difference in growth dynamic of parboiled effluent (PE) and yeast malt (YM) were observed. From 4 to 24 h, similar growth rate was observed in both media. *S. cerevisiae* maintained the rate in PE media from 24 to 72 h in a higher level than in YM that remained in stationary phase until the end of experimental period (72 h). The mean highest number of viable cells obtained were 2 x 10^{11} CFU mL^{-1} at 72 h for PE and 2.8 x 10^8 CFU mL^{-1} at 72 h for YM (Fig. 1). *S. cerevisiae* biomass was evaluated until 72 h of culture and the results are presented in Figure 1. The highest increase of biomass in PE occurred between 12 and 24 h (66.25%), followed by 36 to 48 h (27%), 48 to 72 h (13.4%) and 24 to 36 h (9.3%). The highest concentration of biomass was detected in PE at 72 h and reached 8.42 g L^{-1}. The biomass in control media remained lower than in PE over the 72 h of culture and reached a maximum of 4.9 g L^{-1} at 48 h.

Environmental parameters

Table 1 presents the removal rates (%) of total nitrogen (TKN), chemical oxygen demand (COD), and phosphorus (P) in PE inoculated with *S. cerevisiae*, estimated as follows:

$$\%removal\ rate = \frac{100 \times (Initial\ conc. - Final\ conc.)}{Initial\ conc.} \qquad (3)$$

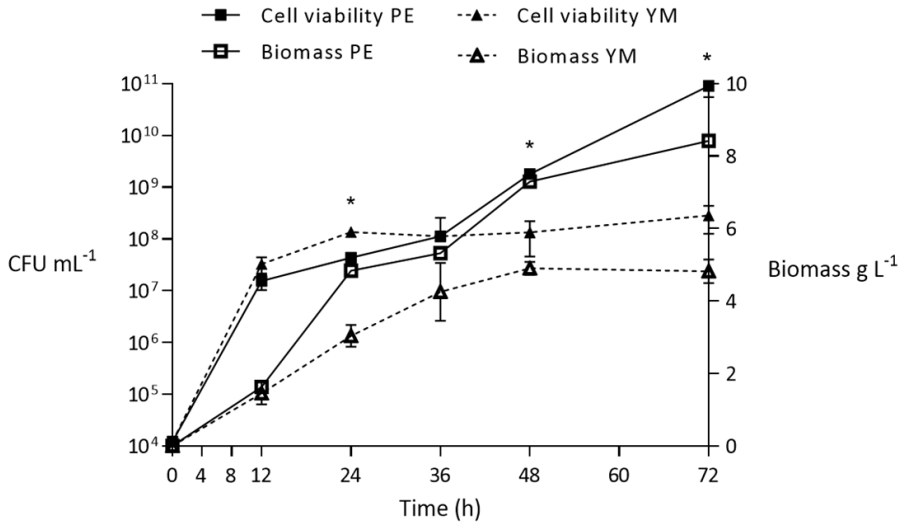

Fig. 1. *Saccharomyces cerevisiae* biomass and growth in PE and YM media. The data represents the mean (+/-SD) of each parameter at 0, 12, 24, 36, 48 and 72 h of culture. Asterisks (*) means statistical significance ($p \leq 0.05$) between PE and YM results for cell viability and biomass

Samples at 0 and 48 h were analysed to verify the potential of *S. cerevisiae* in reducing the environmental parameters in PE. Statistical significance ($p<0.05$) was observed for all parameters.

DISCUSSION

The rice processing at the parboiled rice industry generates high volumes of a non-valued effluent that is directly treated at effluent treatment stations. Cultivating *Saccharomyces cerevisiae* in PE we are adding value to this productive chain and producing biomass that can be used as a co-product. The high cell viability observed in the present study allows the application of *S. cerevisiae* as probiotic (Suarez and Guevara, 2018), replace the protein source in animal food (Broadway et al. 2015; Lara-Flores et al. 2003; Rumsey et al. 1991), and as source of nutrients to culture microorganisms (Champagne et al. 2003; Rakin et al. 2004). The urgent demand for new treatment technologies to maintain the sustainability and competitivity levels of parboiled rice industry is required to reduce the actual minimum retention time of ±15 days at PE treatment plant.

Table 1. Removal rates (%) of TKN, COD and P in parboiled effluent inoculated with S. cerevisiae. Standard deviations are presented by ± SD

Medium	%TKN	%COD	%P
PE	73.45 ± 5.75	55.96 ± 1.17	17.28 ± 1.13

S. cerevisiae is one of the most adapted and studied yeasts, widely employed on bioremediation, since it can metabolise nutrients from complex matrices and is resistant to variations (Divya et al., 2015; Rodrigues Reis and Hu, 2017). Our working hypothesis was that culturing *S. cerevisiae* in PE we would be able to reduce the environmental parameters of TKN, COD, P, and generate yeast biomass of high viability as co-product from treatment. PE supported the nutrient requirements of *S. cerevisiae* and resulted in growth with high cell viability, with kinetics comparable to the YM media from zero to 36 hours with no detectable adaptation phase (Figure 1). The logarithmic growth from 0 to 12 h in both cultures indicates the consumption of simple carbon sources as this carbohydrate was present in both medias. This hypothesis was also supported by the kinetic of growth observed in PE from 36 to 72 h. The higher cell viability in PE after 36 h and statistical significance between the averages of each media at 48 and 72 h showed that PE can provide enough nutrients to overcome a well stablished standard media such as YM. Gaboardi et al. (2018) reported the consumption of approximately 90% of sucrose on first 6 hours of *Saccharomyces* growth in PE, leading to a reduced growth rate. One may suggest that what happen in PE culture was a mechanism of diauxie (Wang et al. 2019), regulatory mechanism where only the enzyme for the preferred carbon source is expressed, even when multiple sources are present (Gancedo 1998). In YM the carbon source is

glucose, and glucose is also present in PE, but since PE is a complex media, different carbon sources may be present promoting diauxie effect. However, this hypothesis was out of the scope in this study.

The biomass (Figure 1) in PE remained higher than in control media YM over the 72 h of culture and followed the increasement on cell viability with the highest growth from 0 to 12 h, steady from 12 to 36 and returning to increase from 36 to 72 h. The simple culture setup presented on this study using baffled flasks with no air injection and supplementation, reached a notable biomass of 8.42 g L^{-1} after 72 h. One may suggest that approximately 6.300 kg of *S. cerevisiae* biomass could be produced daily from an average volume of effluent of 750 m^3, considering the capacity of production of our local rice industries. Culturing in bioreactor and supplementing PE with 1% of sucrose, *S. cerevisiae* var. *boulardii* was able to grow and reached 3.8 g L^{-1} of biomass (Gaboardi et al. 2018). Supplementing parboiled rice effluent with 15 g L^{-1} of biodiesel-derived glycerol, Gil de los Santos et al. (2012) obtained 2.1 g L^{-1} with the yeast *Pichia pastoris* X-33. Combining an enzymatic pre-treatment of parboiled rice, Fehrenbach et al. (2021) reported a 2 g L^{-1} biomass in 48 h of culture and air injected cultures of 4 L. According to the aforementioned, the biomass results in this study can be later improved by an optimization of culture conditions.

The average concentrations of TKN, COD and P in raw PE at 0 h were 137.5, 574.5 and 150 mg L^{-1}, respectively. The potential use *S. cerevisiae* in the treatment of PE was observed in the high removals of 73.45% in TKN, 55.96% in COD and 17.28% in P levels. Industrial and domestical effluents are discharged in the environment after successive treatment steps to adequate the effluent to discharge limits, usually stablished by the state environmental agency. This study was developed in Rio Grande do Sul, the main producer of rice in Brazil and main exporter of parboiled rice. The discharge limits in the state are stablished by CONSEMA, currently more rigorous than the federal limits. On this way, a biological alternative to assist the effluent treatment stations in reaching the removal levels is urgently required. In this study, the culture with *S. cerevisiae* allowed the removal of TKN in 15 mg L^{-1} higher than the stablished by CONSEMA (20 mg L^{-1}), 47 mg L^{-1} under the concentration for COD (300 mg L^{-1}), 117 mg L^{-1} and 57% higher for P (75% or 3 mg L^{-1}). To note, the nutrients were not only removed from the PE but converted in to biomass, highlighting the nutritional level of the coproduct. If operated in conjunction with the current effluent treatment stations, the nutrient levels can be possibly reduced under the state limits. Moreover, *S. cerevisiae* showed robustness and maintained proportional removals, high cell viability and was not negative influenced by variations in pH, COD, TKN and P in PE. These variations were also reported by Queiroz and Koetz (1997) and Mukherjee et al. (2016) and occur mostly due to seasonally and process variations. On this scenario, the microorganism had to tolerate, endure and adapt to maintain its industrial applicability. In this study we observed that PE allowed the growth of *S. cerevisiae* without supplementation, reducing the environmental parameters and producing a yeast biomass of high cell viability as product. *S. cerevisiae* may be used as a method to reduce cost and to add value for rice parboiling effluent.

CONCLUSION

In this study we investigated the bioremediation potential of *Saccharomyces cerevisiae* YT001 for the treatment of rice parboiled effluent. We were able to obtain removals of 74% of TKN, 55% of COD, and 17.28% of phosphorus, followed by high cell viability of $\pm 2\times10^{11}$ CFU mL^{-1}, and 8.4 g L^{-1} of biomass. These results suggest that *S. cerevisiae* is a promising alternative to assist the nutrient removal from PE and generate a marketable biomass.

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