BIOTREATABILITY OF WATER IN CLOSED-LOOP SYSTEMS OF THE RECYCLED PAPER INDUSTRY

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ABSTRACT

The pulp and paper sector are essential to Brazilian economy, particularly units focused on recycled paper production. Waste and inputs used during the industrial process generate contaminants that affect water quality, especially in plants without effluent discharge. As a sustainable approach, fungal cultivations have been employed to mitigate these effects in an increasing number of industries. This study aimed to improve water quality in a closed-loop recycled paper mill. Cultivations of *Pleurotus albidus* and *Lentinus crinitus* were carried out in a medium containing 90% v/v of recycled water with glucose concentrations ranging from 0 to 20 $g.L^{-1}$. Significant reductions in color values of 85% and 59% were achieved in *P. albidus* and *L. crinitus* cultivations, respectively. Additionally, electrical conductivity showed about a 30% reduction for both fungal cultures, even under specific cultivation conditions. These results demonstrate the feasibility of applying fungal cultures to improve water quality in paper mills through sustainable practices.

Keywords: Fungal cultures. Enzymes. Recycled paper. Closed-loop systems. Sustainability.

1 INTRODUCTION

With an increasing focus on sustainability, various industries are adopting measures to reduce resource consumption and minimize environmental pollution[1]. Recycling is being widely explored, especially in the paper industry, where closing water circuits is a common practice to reduce the intake of fresh water. However, this closure can lead to contamination problems due to the presence of organic and inorganic contaminants in the generated effluents[2]. To mitigate these issues, treatment strategies are needed, including biotechnological methods which utilize lignolytic enzymes, particularly those obtained from cultures of basidiomycete fungi such as the genus Pleurotus and Lentinus[3,4]. These enzymes not only act in contaminant reduction but also have significant potential for various industrial applications aligned with Sustainable Development Goals (SDGs), such as those related to industrial recycled paper innovation and responsible consumption[5]. This study aims to evaluate these biological strategies to improve water quality in closed-loop recycled paper production circuits.

2 MATERIAL & METHODS

The fungal cultures utilized were *Pleurotus albidus* 93F and *Lentinus crinitus* 20 M-AS belonging to the culture collection of the Enzymes and Biomass Laboratory (LENB) at the Institute of Biotechnology, University of Caxias do Sul (UCS/RS). The cultures were maintained on Potato Dextrose Agar (PDA) medium at 4°C.

The recycled water used in the experiments was provided by a recycled paper packaging manufacturing company with a closed-loop water system located in the Serra Gaúcha region (RS). Samples of the recycled water were stored in 5-liter polypropylene containers and kept under freezing conditions (-18°C) until further use. Physicochemical analyses were carried out at the Laboratory of Environmental Analysis and Research (LAPAM) of the University of Caxias do Sul, using standard procedures (Standard Methods for Examination of Water and Wastewater, 2017, 23rd edition). In addition, during the cultivation, some parameters such as electrical conductivity and apparent color were also experimentally determined at the laboratory of the industry that supplied the recycled water.

Fungal cultures were initially transferred to PDA agar medium and incubated for 120 hours at 28°C. Subsequently, four (4) discs with a diameter of 1.5 cm were transferred to Erlenmeyer flasks for biomass obtainment. The medium described in Kirsch (2013) containing glucose, yeast extract and mineral salts was used[6]. Erlenmeyer flasks containing 100 mL of medium with an initial pH adjusted to 6.0 were sterilized at 1 atm for 15 minutes. Subsequently, these flasks were inoculated and kept under growth conditions on a benchtop shaker at 180 rpm, 28°C for 120 hours. At the end of the cultivation, the total volume of the media was mixed in a pre-sterilized flask within a laminar flow hood, and this mixture was used as vegetative inoculum for subsequent tests.

The water treatability assays were performed considering the effect of the percentage of recycled water (90% v/v) and initial glucose concentration (0 to 20 g.L-1) on fungal growth and improvement of recycled water quality. The respective formulated media (100 mL), initial pH adjusted to 6.0, were transferred to 500 mL Erlenmeyer flasks and sterilized at 1 atm for 15 minutes. Subsequently, the media were inoculated with 10% (v/v) of the vegetative culture of *P. albidus* or *L. crinitus* previously grown for 120 hours, and the flasks were sealed with gauze and hydrophobic cotton. The cultivation time was 12 days, at 28°C, and 180 rpm. Samples were taken from destructive flasks at the initial time (0 hours) and subsequently every 3 days of cultivation. The

experiments were carried out in triplicate, and the results were evaluated using analysis of variance (ANOVA) followed by Tukey's post hoc test, with a probability level of less than 5% ($p < 0.05$).

Cell growth was determined gravimetrically. The determination of glucose concentration was performed using enzymatic kit (Laborclin, SP, Brazil), at 505 nm. The laccase activity was determined using ABTS substrate, at 420 nm[7]. The quantification of apparent color was evaluated at 465 nm[8].

3 RESULTS & DISCUSSION

The physicochemical parameters of recycled water are shown in Table 1. Since the industry only recycles and does not discharge the water used in the process, there are no established legal limits for the mentioned parameters, and also no regulations for discharges into water bodies. However, high values of electrical conductivity, chemical oxygen demand, biochemical oxygen demand, and hardness can be observed. These parameters influence the performance of the process causing the degradation of active chemical components and hindering, to some extent, the formation and drainage of paper sheets.

Table 1 Physicochemical characterization of water sample from recycled paper production process

Test	Result
Biochemical Oxygen Demand (BOD)	21955.9 mg O_2. L^{-1}
Electrical Conductivity (25°C)	15.1 mS/cm
Apparent Color	1000.0 UC
Chemical Oxygen Demand (COD)	42565.0 mg O_2. L^{-1}
Total Hardness	11850.0 mg $CaCO_3$. L^{-1}
Total Dissolved Solids	45982.0 mg.L^{-1}

The values presented in Table 1 cannot be directly compared with data from specialized literature because most recycled paper mills have open-loop water systems. Therefore, there are no reports regarding the water quality of industries practicing closed-loop water recycling. Furthermore, it should be emphasized that the quality and components present in recycled water vary depending on the paper scraps and captured water used for paper production, resulting in an unstable or heterogeneous recycled water[9].

The cellular growth profiles of *P. albidus* and *L. crinitus* and the variation in glucose concentration during the cultures are presented in Figure 1. It can be observed that even in the absence of glucose, the cell growth was similar to that identified by using 5 $g.L^{-1}$ and 10 $g.L^{-1}$ of substrate (Figure 1A and Figure 1B). At higher concentrations, 10 $g.L^{-1}$ and 20 $g.L^{-1}$, the growth profile of *P. albidus* was slower for up to 6 days. At the end of cultivation, approximately 11 $g.L^{-1}$ and 16 $g.L^{-1}$ of biomass for *P. albidus* and *L. crinitus* were reached respectively. In terms of glucose metabolism, residuals of 2.0 $g.L^{-1}$ and 50 $g.L^{-1}$ were quantified in media formulated with 15 and 20 $g.L^{-1}$ of glucose. Furthermore, residual glucose of 11 $g.L^{-1}$ was observed in *L. crinitus* cultivation in a medium composed of 20 $g.L^{-1}$ of glucose (Figure 1C and Figure 1D).

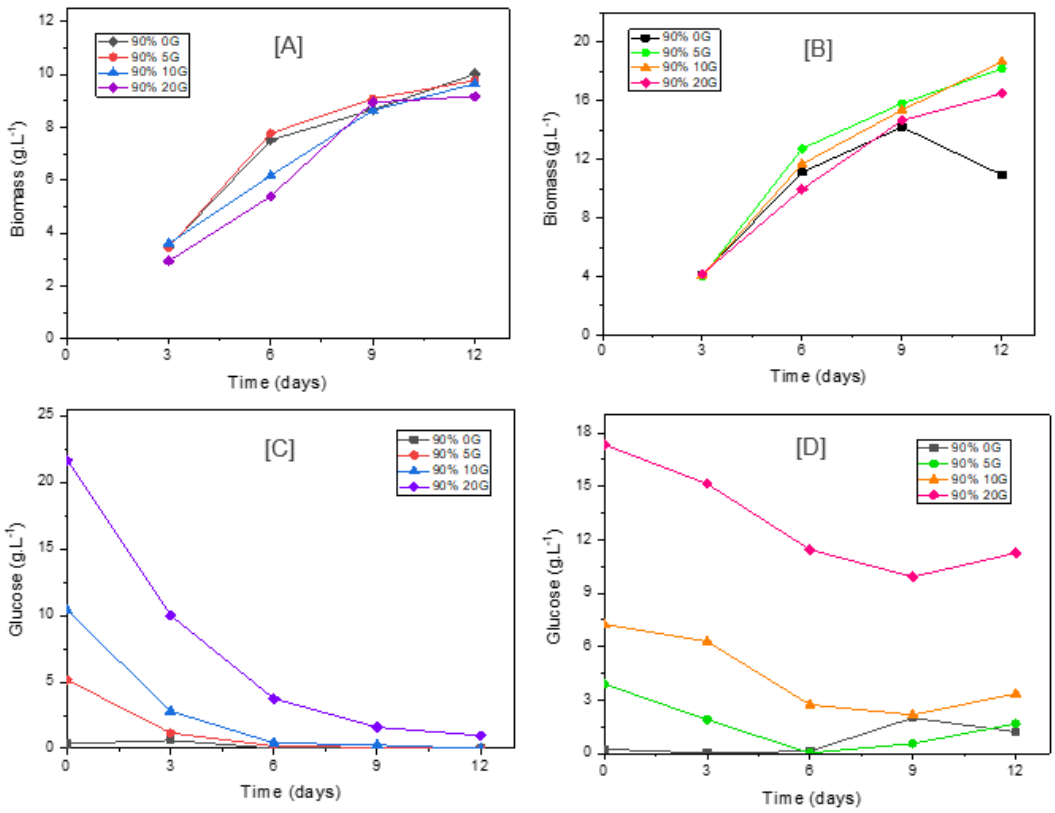

Figure 1 Variation of biomass production [A] and glucose consumption [C] as a function of time of *Pleurotus albidus* cultivation and biomass production profile [B] and glucose consumption [D] as a function of time of *Lentinus crinitus* cultivation.

Regarding the color determination, changes can be observed due to the effect of microbial growth itself. As the fungal growth behavior exhibited a gradual response to the glucose concentration in the medium, no statistically significant differences were identified in the color reduction after 12 days of *P. albidus* and *L crinitus* cultivation, being attained average around 85% and 59%, respectively. The color reduction during the process suggests the degradation of chromophore compounds present in the recycled water by the microbial population. These behaviors could be advantageous for industrial plants, as the color of water can impact the final product or certain steps of the production process[10].

During *P. albidus* and *L. crinitus* cultivations, an average reduction of 30% in electrical conductivity was identified when compared to the initial time of process and in all glucose concentration evaluated. The reduction in electrical conductivity of the recycled water may be correlated with the microbial degradation of salts and other chemical compounds present into the medium. With the consumption of these compounds, the electrical conductivity is reduced, favoring the recycled water decontamination. The decrease in electrical conductivity enhances the performance of sizing agents and other chemical inputs used in paper machines, as a high concentration of salts interferes with the performance of these inputs, degrading their active components[11].

Additionally, the activity of laccase enzymes was assessed in both cultivations. Even though distinct values were measured in *P. albidus* and *L. crinitus* cultivations, with higher values in the latter case, the results are consistent with a significant decrease in sample coloration. The production of laccases, evaluated without specific inducers, suggests the constitutive secretion of the enzymes as an important factor in terms of water treatability for recycling. It is important to note that the conditions for catalytic stability of these enzymes over time and under different storage conditions were not determined.

In a global perspective, considering the results of color reduction and electrical conductivity in the fungal cultivation, it can be suggested that a 9-day process period would be sufficient to achieve significant outcomes in terms of recycle water treatability from the recycled paper industry. This would also result in operational cost reduction. The results demonstrate the efficiency and importance of clean technologies development in industrial processes.

4 CONCLUSION

The tolerance of *P. albidus* and *L. crinitus* to the compounds present in recycled water was identified, considering its growth profile and carbon source consumption during the cultivation. The electrical conductivity reduction in the medium containing 90% of recycled water demonstrates the potential application of the biological process in the industry. However, this parameter could be enhanced through further studies on the optimal cultivation conditions. For industries in which water coloring could cause changes in the production process, a sustainable alternative for the reduction of this problem is evidenced. The developed studies offer new alternatives for water and effluent treatment in the paper industry. However, for industrial application, further steps need to be undertaken and scaled up.

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