



COUPLING ANAEROBIC DIGESTION AND WHITE-ROT FUNGI FOR SOLUBLE COFFEE WASTEWATER TREATMENT

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Highlights

- Fungal treatment enhances the removal of complex pollutants after anaerobic digestion.
- *Pleurotus ostreatus* improves post-treatment of soluble coffee wastewater.
- Efficient removal of phenolic compounds and color by fungal treatment.

Introduction

Coffee is one of the most widely consumed beverages in the world, and its production chain—from fruit processing to soluble coffee manufacturing—generates large volumes of wastewater. During grain processing, coffee processing wastewater (CPWW) is produced, while soluble coffee production generates soluble coffee wastewater (SCWW). CPWW shows high chemical and biochemical oxygen demand (COD and BOD), whereas SCWW, though darker in color, contains lower organic matter. Both, however, contain compounds such as polyphenols, tannins, polysaccharides, and caffeine, which hinder natural degradation and inhibit microbial growth.

Anaerobic digestion has proven to be an effective alternative for treating wastewater generated in the coffee production chain, such as CPWW (PIN et al., 2020) and SCWW (MOSQUERA et al., 2019). This process is valued for its low energy consumption, reduced sludge production, and conversion of organic matter into biogas (PENG et al., 2020). However, toxic compounds, especially phenolic compounds, can negatively interfere by inhibiting critical stages of digestion and reducing treatment efficiency (MONLAU et al., 2014). Alternatively, white-rot basidiomycete fungi, such as *Pleurotus ostreatus*, known for their ability to grow in harsh environments where prokaryotes struggle, play a key role in lignin degradation through the release of extracellular enzymes, converting it into CO₂ and H₂O (SCHWARZE, 2007).

Given the environmental challenges posed by the wastewater from the soluble coffee industry, particularly due to the high concentration of toxic phenolic compounds, this study aimed to evaluate the potential of the basidiomycete fungus *Pleurotus ostreatus* for post-treatment of this wastewater following anaerobic treatment in an ASBBR reactor. The focus was to assess the fungus's ability to promote decolorization, degrade phenolic compounds, and reduce the associated toxicity, thereby enhancing the overall treatment efficiency of these complex wastewaters.

Material and Methods

Laboratory scale ASBBR

An anaerobic sequencing batch biofilm reactor (ASBBR) with a working volume of 4 L was constructed, featuring a stainless-steel basket containing polyurethane cubes (1 cm) for biomass immobilization. Sludge granules were macerated and mixed with 65 g of support material, allowing 24 h for adhesion before placement. During immobilization, the reactor was fed with glucose-based synthetic effluent and operated at 30 ± 1 °C in decreasing cycle durations. After this phase, it was fed with soluble coffee wastewater (SCWW) at an OLR of 2.5 kg/m³/day, operated in 24-hour cycles with 50% exchange, mechanical stirring at 500 rpm, and maintained at 35 ± 1 °C.



Post-treatment of ASBBR by *Pleurotus ostreatus*

The post-treatment of the ASBBR effluent using *Pleurotus ostreatus* was carried out in two stages. In the first test, the fungus was evaluated for its tolerance to different effluent concentrations (25%, 50%, 75%, and 100%). Initially, the dilutions were made with distilled water, but fungal growth was unsatisfactory due to carbon limitation. Therefore, the concentrations were re-evaluated using CPWW as the diluent. In the second stage, the best condition identified was used for a kinetic study of the post-treatment, conducted over 18 days with daily sampling. The experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL of medium at pH 6.5, 28 °C, and 120 rpm (Silva, 2022).

Analytical techniques

The analyzed parameters included COD, carbohydrates, pH, total solids (TS), volatile solids (VS), fixed solids (FS), and total phenolic compounds (expressed as gallic acid equivalents), according to APHA (2012), Blundi & Gadelha (2001), and Bravo et al. (2013). Short-chain volatile fatty acids were quantified by gas. Laccase activity was determined by the oxidation of ABTS at 420 nm in 0.1 M sodium acetate buffer (pH 4.8), according to Ballaminut and Matheus (2007), with one enzymatic unit defined as the amount of enzyme capable of oxidizing 1 μmol of substrate per minute.

Results and Discussion

The SCWW and CPWW wastewaters were characterized based on their physicochemical parameters, and the results are presented in Table 1.

Table 1 Physicochemical parameters of wastewaters.

Parameters	SCWW	CPWW
COD (mg L^{-1})	14,500.0	10,628.50
TPC (mg L^{-1})	866.4	27.20
Total sugars (mg L^{-1})	1,486.0	9,196.52
pH	7.70	4.16
Total solids (TS) (mg L^{-1})	9,357.0	64.36
Fixed solids (FS) (mg L^{-1})	5,309.0	7.64
Volatile solids (mg L^{-1})	4,048.0	56.72

Table 2 presents the removal percentages of total phenolic compounds and COD. The reactor demonstrated efficient removal of organic matter, maintaining a relatively stable performance over time. The maximum removal efficiency of soluble COD reached approximately 60%, which is lower than the approximately 80% reported by Chernicharo (1997) for anaerobic reactors. However, the reactor's efficiency in removing phenolic compounds was unsatisfactory, achieving only 19.7% removal. This limited performance may be attributed to the high concentration of phenolic compounds in the effluent, which are potentially toxic (Veeresh et al., 2005) and can inhibit the microbial consortium responsible for anaerobic digestion (Monlau et al., 2014; Novita, 2016), thereby compromising the overall treatment efficiency (Chapleur et al., 2016).

Table 2 Average values of the monitoring parameters of the ASBBR reactor.

Parameters	ASBBR Influent	ASBBR Effluent
COD (mg L^{-1})	5190.73	2274.40
TPC (mg L^{-1})	348.87	280.40
COD removal efficiency (%)		56.18
Phenolic compounds removal (%)		19.62
Total alkalinity (mg L^{-1})	1959.41	3354.72
Bicarbonate alkalinity (mg L^{-1})	929.02	3176.02
pH	7.04	8.84
Acetic acid (mg L^{-1})	1459.2	144.2
Propionic acid (mg L^{-1})	2051.7	7.5
Isobutyric acid (mg L^{-1})	9.4	Nd
Butyric acid (mg L^{-1})	189.8	19.0
Isovaleric acid (mg L^{-1})	33.0	20.3

Table 3 presents the results of the tolerance test of *P. ostreatus* to the ASBBR reactor effluent in a liquid medium at different concentrations. The highest removal of total phenolic compounds (TPC) was achieved with 50% effluent (80.5%) (Figure 4), which also showed good removal values for COD (62.2%) and decolorization (18.67%), despite low laccase activity at 15 days. The 100% and 75% concentrations exhibited moderate removal of phenolic compounds but low COD removal at 8.38% and 5.37%, respectively. Although the 25% concentration demonstrated good COD removal, this test contained a small amount of ASBBR effluent and showed lower decolorization and TPC removal compared to the 50% concentration test. Therefore, the 50% concentration was selected for future studies due to its balanced performance.

Table 3 Tolerance test of *P. ostreatus* to ASBBR effluent.

Efluente ASBBR (%)	Laccase (U L ⁻¹)	COD Removal (%)	TPC Removal (%)	Biomass (mg L ⁻¹)	Discoloration (%)
25	6.69	71.95	68.59	2710	15.54
50	3.05	62.24	80.51	1876	18.67
75	25.38	8.38	36.32	570	5.36
100	0.18	5.37	39.15	330	3.08

The COD showed a reduction similar to that observed for carbohydrates (Figure 1a), with a decrease of 59.16% on the 10th day of incubation and 62.72% on the 18th day. The COD profile during the treatment of wastewater by basidiomycete fungi is still poorly studied. Some studies report COD reductions in the treatment of complex wastewaters, such as olive oil production effluents, with removals ranging from 12 to 47% by *P. ostreatus* (Fountoulakis et al., 2002; Ntougias et al., 2015). Biomass showed a strong correlation with the consumption of COD and carbohydrates, with a tendency to increase until the ninth day of incubation, reaching 1936 mg L⁻¹. After this period, there was a decrease in fungal growth and organic matter removal.

In Figure 1b, an inverse relationship is observed between the concentration of total phenolic compounds (TPC) and the increase in laccase activity, with the removal of 107 mg L⁻¹, approximately 70% of the TPC, after 9 days of incubation. After this period, TPC removal stabilizes, reaching a total removal of 83% by day 18. This stabilization may be associated with laccase activity, which peaked on day 7 at 101 U L⁻¹. After this peak, a sharp decline in laccase activity occurs. One possible explanation is the abrupt drop in pH starting on day 6 to about 2.45, likely due to the synthesis of organic acids such as oxalic acid, whose production by *Pleurotus ostreatus* has been demonstrated (Shimad et al., 1996; Yang et al., 2017; Unuofin et al., 2019). Oxalic acid can act by chelating unstable Mn³⁺ ions, supplying H₂O₂, and lowering the pH of the medium. These factors are crucial for the performance of peroxidases, enzymes responsible for degrading lignin and other phenolic compounds (Makela et al., 2002).

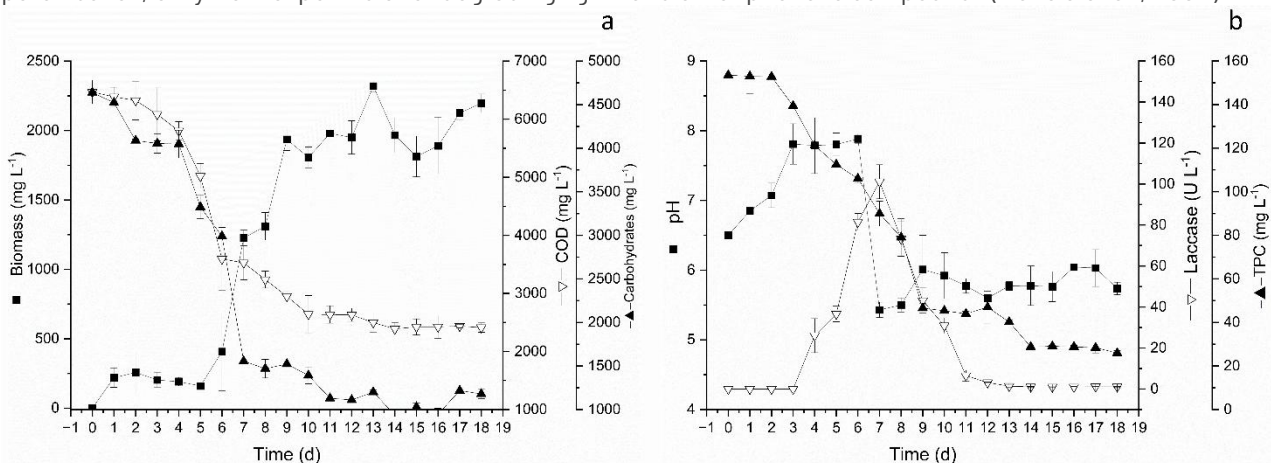


Figure 1 (a) Fungal growth, organic matter consumption in terms of COD, and carbohydrate consumption. (b) Laccase production, pH variation, and degradation of total phenolic compounds by *P. ostreatus*.

Figure 2 shows that the post-treatment with *Pleurotus ostreatus* promoted a progressive reduction in the absorbance of the effluent treated in the ASBBR reactor over 18 days. The most significant decrease occurred between 340 and 600 nm, indicating the degradation of phenolic compounds and humic substances. The visual change in the effluent color, from dark brown to light yellow, reinforces this transformation. These results highlight the ability of the fungus *P. ostreatus* to promote



color removal and possibly degrade complex organic compounds present in the effluent. According to Ferrari and Mingazzini (1995), the range of 310–370 nm indicates structures with two aromatic rings; between 370–400 nm, there is an association with fulvic acid; and above 470 nm, the presence of humic acid is observed.

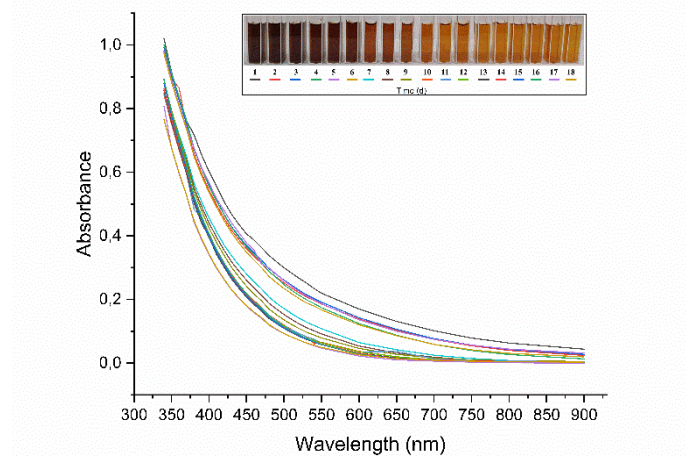


Figure 2 Decolorization of the effluent post-treated by *P. ostreatus*.

Conclusion

This study demonstrates that although the ASBBR reactor provides moderate removal of organic load, its efficiency in reducing toxic phenolic compounds is limited. However, the combination of ASBBR with a fungal post-treatment using *Pleurotus ostreatus* significantly improves the removal of phenols and COD and promotes effluent decolorization. The fungal enzymatic activity, especially that of laccase, plays a key role in this improvement. These results highlight the potential of integrating anaerobic digestion with fungal biological treatment to enhance the quality of complex wastewaters, such as those generated from soluble coffee production.

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