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Combination of catalytic ozonation and fungal bioremediation for treatment from effluent from the laminate production industry

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ABSTRACT

This study characterized and evaluated the potential of a combination of treatment strategies for the removal of phenolic compounds, color, and ecotoxicity of an effluent generated by a laminated wood industry. The characterization of a sample of the effluent generated by the pine lamination process collected in an industry in the Southwest region of the state of Paraná confirmed the potential impact of the effluent, with emphasis on the high content of phenolic compounds (1,530 ±50 mg/L), color (2,159 ±30 units), significant acute toxicity (63% mortality of microcrustaceans *Artemia salina*) and low biodegradability (BOD/COD=0.53), which, if eventually released into receiving bodies, can cause irreversible and harmful effects to the aquatic microbiota. The treatment processes studied in this work involved catalytic ozonation mediated by ferrous catalytic material based on steel scale and biological process with *Pleurotus Florida* fungi, evaluated in an individual way and sequentially integrated as follows: Catalytic ozonation>>biological process and vice-versa. The best results were obtained by applying the sequence "catalytic ozonation>>biological process", which resulted in reductions of 96.2% in soluble lignin concentrations, 93.3% in color units and more than 99% reduction in total phenols, in addition to a low mortality rate of *A. salina* (≈10%). In the integrated process, the application of catalytic ozonation before the biological process presents greater advantages because of the pre-decomposition process of recalcitrant compounds by the chemical action of catalytic ozonation, which can convert these compounds into biodegradable, facilitating the action of the biological process for the remediation of the effluent.

Keywords: fungi, ozonation, phenols, wood lamination.

Combinação de ozonização catalítica e biorremediação fúngica para tratamento de efluente da indústria de produção de laminados

RESUMO

Este estudo foi voltado a caracterização e avaliação do potencial da combinação de estratégias de tratamento para a remoção de compostos fenólicos, cor e ecoxicidade de um efluente gerado por uma indústria madeireira de Produção de Laminados. A caracterização de uma amostra do efluente gerado pelo processo de laminação de pinus coletada em uma indústria da região Sudoeste do estado do Paraná confirmou o potencial impactante do efluente, com destaque para o elevado teor de compostos fenólicos (1.530 ±50 mg/L), cor (2.159 ±30 unidades), significativa toxicidade aguda (63% de mortalidade de microcrustáceos *Artemia. salina*) e baixa biodegradabilidade (DBO_5/DQO=0,53) podendo, se eventualmente lançado em corpos receptores, causar irreversíveis e deletérios efeitos a microbiota aquática. Os processos de tratamento estudados neste trabalho envolveram ozonização catalítica mediada por material catalítico ferroso baseado em carepas de aço e processo biológico com fungos *Pleurotus florida*, avaliados de forma individual e integrados da sequinte forma: Ozonização catalítica>>processo biológico e vice-versa. Os melhores resultados foram obtidos pela aplicação da sequência "ozonização catalítica>>processo biológico", que resultou em reduções de 96,2% em concentrações de lignina solúvel, 93,3% nas unidades de cor e mais de 99% na redução de fenóis totais, além de baixa taxa de mortalidade de *A. salina* (≈10%). No processo integrado, a aplicação da ozonização catalítica antes do processo biológico apresenta maiores vantagens em razão do processo de pré-decomposição de compostos recalcitrantes pela ação química da ozonização catalítica, que pode converter estes compostos em biodegradáveis, facilitando a ação do processo biológico para a remediação do efluente estudado.

Palavras-chave: fenóis, fungos, laminação de madeira, ozonização.

1. INTRODUCTION

In the laminate production industry, the effluent generated by cooking wood prior to mechanical processing in order to obtain sheets contains a variety of chemical compounds that make up the organic load of wood, such as cellulose, hemicellulose, and lignin, in addition to extracts such as essential oils, resins, phenols, tannins, fatty acids, coloring, and terpenes. This variety of compounds, particularly lignocellulosic and phenolic compounds, increase the potential impact of the effluent, which may add low biodegradability and significant acute toxicity, so that when released without treatment it can cause harmful physicochemical changes to the receiving water bodies (Feron, 2016).

The processes generally used for remediation of these effluents, represented by conventional technologies such as stabilization ponds or aerated ponds, have a questionable application, mainly due to their low efficiencies and the need for long hydraulic detention times (Von Sperling, 1996; Litchfield, 2005; Huang *et al.*, 2017). On the other hand, alternatives such as processes that involve membrane filtration or even adsorption with activated carbon, despite having significant efficiency for removal of organic matter, contaminants, suspended solids, and microorganisms, still need to overcome technical challenges such as incrustation, saturation and final disposal of contaminated materials, increasing operating and maintenance costs (Im, *et al.,* 2019).

Catalytic ozonation belongs to the class of advanced oxidative processes (POAs), recognized for its high efficiency in the effective degradation of refractory, chemical compounds, which can, under optimized conditions, lead to mineralization or promote levels of degradation sufficient to reduce acute toxicity and raise biodegradability (Malik *et al.*, 2020). Although the low selectivity of the oxidizing species constituting the POAs (hydroxyl radical) makes its applications in more complex matrices difficult, they can represent valuable alternatives to either be used in combination with conventional processes, as pre-or post-treatment, to elevate the efficiency of secondary treatment or complement it through the degradation of residual refractory chemical species (Rekhate and Srivastava, 2020).

The enzymatic action in effluent treatments and pollutant removal using biological processes with white-rot fungi is described as an efficient, cheap and safe method that has an action on a wide variety of polluting compounds; laccases and peroxidases are defined as reference enzymes in the function of its efficient capacity for catalytic redox action in the decomposition of multiple diversity of pollutants (Bilal *et al.*, 2021).

In the combined treatment of effluent generated by paper production industries, Silva (2005) evaluated a process that involved an air-lift reactor with white-rot fungi, *Lentinus edodes*, followed by photocatalytic treatment. The biological treatment was carried out using mycelium immobilized on polystyrene threads in an air-lift reactor and showed results with reductions of 34% for total phenols observed at 48 hours and COD (40%) after the 5th day of treatment. When applying the heterogeneous photocatalysis process with 2.4 g/L of TiO_2 at pH = 5.2, in 6 hours of treatment, 86% reduction in color and 67% in COD were verified. When the combined treatments were applied, pre-treatment with fungus followed by treatment by photocatalysis resulted in the highest removals for color (88%), total phenols (75%), and effluent COD (75%).

In this context, this study evaluated the advantages of integrating catalytic ozonation with a biological process operated by white-rot fungi to treat effluent from the wood industry for the production of laminates.

2. MATERIAL AND METHODS

2.1. Chemicals and supplies

All chemicals used were of analytical grade and were obtained from Merck, Reagen, or Sigma. The wastewater samples were collected from Wood Laminate Industry, located in Paraná, Brazil. The wastewater resulted from the wood laminate stage. After being collected at room temperature, it was stored under refrigeration (4ºC) for later analysis.

2.2. Analytical control

The following parameters were used to determine the pollution potential of the effluent and ensure analytic control of the system:

Determining the color: The color was measured according to CPPA standard methodology (1975). All determinations used one (1) mL sample and 250 L phosphate buffer. The samples were previously centrifuged for 15 minutes at 3500 rpm and were then adjusted to pH 7.6 with 0.1 $mol.L^{-1}$ phosphate buffer.

Chemical oxygen demand (COD): A sample refluxed in a strongly acid solution with a known excess of $K_2Cr_2O_7$. The consumed oxygen was measured against the standard at 600 nm with a spectrophotometer (APHA *et al.*, 2005).

Soluble Lignin: The modified method presented by Ruzene (2005) was used, where 5 mL of the filtered sample was subjected to hydrolysis by reducing the pH to 2 ± 0.1 (with 1 $mol.L^{-1}$ H_2SO_4), stirring and heating of 45 ± 0.5°C for 7 min. Subsequently, the reaction was stopped by raising the pH to 12.5 ± 0.1 with 0.1 $mol.L^{-1}$ NaOH, and the absorbance of the hydrolyzate solution was measured at 280 nm, taking the 0.1M NaOH solution as blank. The concentration of soluble lignin present in the sample was obtained through the expression: $[Lig] = (41.87.Abs_{280nm} - 0.3279)\ .10^{-3} g.L^{-1}$ where: [Lig] = Lignin concentration in the hydrolyzate (g.L-1) and $Abs_{280\ nm}$ = Absorbance at 280 nm.

Total Phenols: The total concentration of phenols was determined by the colorimetric method, using the Folin-Ciocalteu (Merck) procedure, which consists of the reduction achieved by the phenols present in the sample to the Folin-Ciocalteu reagent by forming a blue colored complex (APHA *et al.*, 1995).

*Biochemical oxygen demand (BOD_5): W*as carried out using standard methodology with sample incubation, nutrient dilution water, and seed for five days at 20°C.

Wood laminate effluent acute toxicity with Artemia Salina: The procedures followed the NBR 12,713 (ABNT, 2009) and evaluated the acute toxicity of samples from wastewater, surface or subterranean waters and continental soluble chemicals or chemicals dispersed in the water.

Biodegradability Ratio (BOD_5/COD): The biodegradability ratio was obtained by dividing the value of the biochemical oxygen demand (BOD_5) of oxygen and the chemical oxygen demand (COD) of the effluent, aiming to verify the biodegradability of the effluent under study.

2.3. Effluent treatment process

Microorganism and inoculum preparation: The ligninolytic fungus *P. florida* PSP 1 (Fungibrás/SP) was initially grown on potato dextrose agar (PDA) in Petri dishes at 37°C for seven days and then transferred to the liquid culture (Malt Broth, MB) by punching out 5mm of the agar plate with a sterilized self-designed cutter. Twenty disks were used in each flask as standard inoculum.

Biological Wood laminate effluent treatment: After 72 hours, the pre-inoculum was filtered through sterile gauze and, aiming to attenuate the shock load (sensitivity) with the raw effluent, the fungus biomass (20g) was transferred to 250ml of effluent diluted with distilled water (1:200) and subjected to treatment (pH 5,5, 7,9g/L of sucrose, shaker at 200 rpm and 37°C) for 21 days. Samples were collected daily, filtered, and analyzed according to parameters described in 2.2.

Catalytic ozonation with steel scale: The system used a bench-top reactor made of cylindrical-shaped, conical-based borosilicate glass with a capacity of approximately 0.9 L with 400 mL of raw effluent, initial pH 5 ± 0.1, and a 0.35 g/mL aliquot of catalytic material with a size of 100 µm and injection of 1 LPM of O3 produced from dry compressed air, with its excess being expelled from the top of the reactor and trapped with a 5% KI solution. The reaction process required constant aeration/ozonation to allow and maintain a turbulent system keeping about ¼ of the scale load in suspension. The aliquots removed for analytical control were filtered on a 0.45 µm PVDF membrane.

Statistical analysis: For data interpretation, the toxicity results were submitted for analysis of variance, and the comparison of the mean was made by the Tukey test at 5% probability, using the statistical program Sisvar® (Ferreira, 2011).

3. RESULTS AND DISCUSSION

3.1. Characterization of effluent from the plywood production industry

As can be seen in Table 1, characterization of the pine lamination effluent indicated 4009 ± 100 mg $O_2.L^{-1}$ of COD, 607, 69 ± 60 mg $O_2.L^{-1}$ of BOD_5, 2,159 ± 30 color units, 1,530 ± 50 mg/L of total phenols, 28 mg/L^{-1} of soluble lignin, pH = 4.41, the mortality rate of *A. saline* (%) in the order of 63% and biodegradability expressed by the BOD_5/COD ratio of approximately 0.53. It is relevant to note that except [lignin], all parameters were at worrying levels, these concentrations being characteristic for this class of effluent. For example, in similar effluent characterization studies, Heinz *et al.* (2019) observed a COD of 12.192 mg O_2/L; DBO_5 of 8.849 mg O_2/L, biodegradability ratio DBO_5/COD of 0.72, with 8,333 color units and total phenol charge of the order of 1,220 mg/L. Similarly, Aguiar *et al.* (2009) observed similar values with COD of 12.477 mg O_2/L, BOD_5 of 8.450 mg O_2/L, biodegradability ratio BOD_5/COD of 0.6, color 9849 U.C and 358 ± 0.1 mg/L of total phenolic compounds.

The parameters of most significant concern can be represented respectively by color, total phenols, and ecotoxicity, suggesting that an eventual release of this effluent without treatment to the water receiving bodies would contribute to the occurrence of potential damage related to the inhibition of photosynthetic mechanisms, inactivation of microbial flora and mortality of aquatic species. Likewise, the BOD_5/COD ratio of 0.53 proves the low biodegradability of the effluent. It is important to note that when the BOD_5/COD ratio is more significant than 0.60, it suggests reasonably biodegradable wastewater with the possibility of biological treatment. The lower this ratio, the greater the difficulty of applying this treatment type (Leena *et al.*, 2016).

Table 1. Characterization of the pine lamination effluent.

Parameter	Unity	Wood laminating effluent	Legislation
pH	pH unit	4,41± 0,01	5 – 9*1
[Lignin sol.]	$mg.L^{-1}$	28 ± 5	N.R
Total phenols	$mg.L^{-1}$	1.530 ± 50	0.5*2
Color	Color unit	2.159 ± 50	N.R
DQO	$mg\ O_2\ L^{-1}$	4009 ± 100	300
DBO	$mg\ O_2\ L^{-1}$	607,69 ± 60	Rem. de 60%*3
DBO/DQO	unitless	0,53	N.R
A. saline mortality	%	63	N.R

NR: Not Recommended; *1Conama Legislation 430/2011 (CONAMA, 2011); *2Cema legislation 70/2009 (Paraná, 2009); *3Minimal removal efficiency of 60%.

Finally, the characteristics of the effluent understudy indicate the need to apply a treatment capable of promoting an effective change in these parameters of greater environmental relevance, which is also not in line with current environmental legislation.

3.2. Effluent treatability studies from the wood industry for plywood production

Figure 1 illustrates the results obtained in the characterization of the wood lamination effluent after the application of the treatment processes: Chemical (catalytic ozonation), Biological (*P. florida* fungus), Chemical>>Biological, and vice-versa. In addition to these, the adsorbent capacity of the fungus biomass was evaluated to compare the effectiveness of the biological treatment. As can be seen, the best results were obtained by applying the Chemical>>Biological sequence, which resulted in more than 99% reduction in total phenols, reductions of 96.2% soluble lignin concentrations, and 93.3% in units of color, in addition to a low mortality rate of *Artemia Salina* (≈10%). It is possible to observe that the isolated treatments (individual) did not show the same efficiency. In some, there was an increase in the levels of color or phenols (biological and biological>>chemical treatments). A possible justification for this behavior could be the breakdown of molecular compounds with long chains, such as lignin (Fengel and Wegener, 1989).

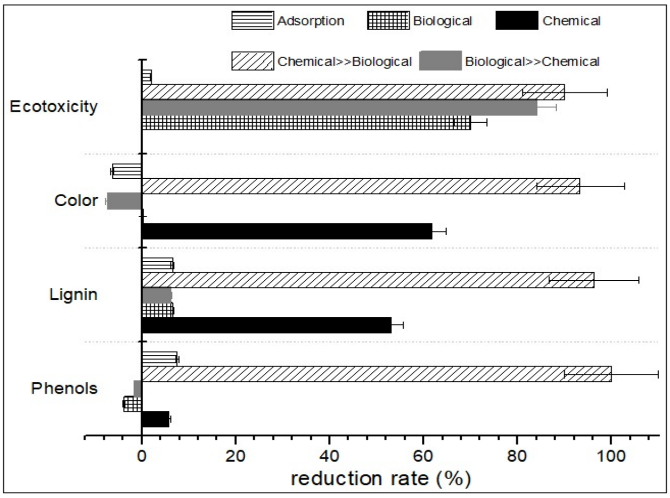

Figure 1. Results obtained in the characterization of the wood lamination effluent after the application of the treatment processes.

Finally, the results obtained by analysis in triplicate allowed us to verify the residual levels (after treatment) of the color parameters, soluble lignin, and phenolic compounds which were respectively, 144 color units, 1,06 mg/L and 0,49 mg/L. These results also show that the treatment attenuated these levels to the values allowed by current legislation. These results also show that the levels of reduction achieved (Figure 1) configure the potential of using this integrated treatment process with the use of catalytic ozonation before the biological process.

It is important to emphasize, however, that the sequence of treatments with the best performance observed in this work diverges from the results reported by Barreto-Rodrigues *et al.* (2009), who studied the treatability of cellulose delignification effluent using chemical (photocatalysis with TiO_2/UV) and biological processes (*Aspergillus* 2BNL1), noted better efficiency in the application of a biological process preliminary to the chemical process, and has been able to reduce color, total phenols, acute toxicity (inhibition of *E. coli* growth) and TOC on the order of 94.2%, 92.6%, 4.9%, and 62%, respectively. However, it is essential to point out that in this work, one of the reasons mentioned by the authors to justify the advantages of this type of combination is the high color of the effluent, which significantly hindered the transmission of radiation necessary for the activation of TiO_2 in the photocatalytic treatment.

Among the possible reasons that could help explain the better efficiency of the combined Chemical,>>Biological process are: a) Advanced oxidation by catalytic ozonation of phenolic compounds and lignin derivatives, and this process also led to a significant color reduction, as indicated by the chemical process in Figure 1; b) The increase in biodegradability and dissolved oxygen concentrations produced by the reaction of ozone with ferrous catalytic material after chemical treatment (Yang *et al.*, 2020; Huang *et al.*, 2017).

Figure 2 illustrates the effects of the different treatments studied on the mortality of *A. salina* (Table 2). The data variability was represented by the error bars in the graph, in which it is possible to verify that the chemical treatment and the integrated Chemical x Biological treatment promoted the highest and lowest mortality rate, on the order of 100 and 10%, respectively. Additionally, the Tukey test indicated that the integrated treatments promote lower *A. salina* Nauplii mortality rate than the isolated treatments.

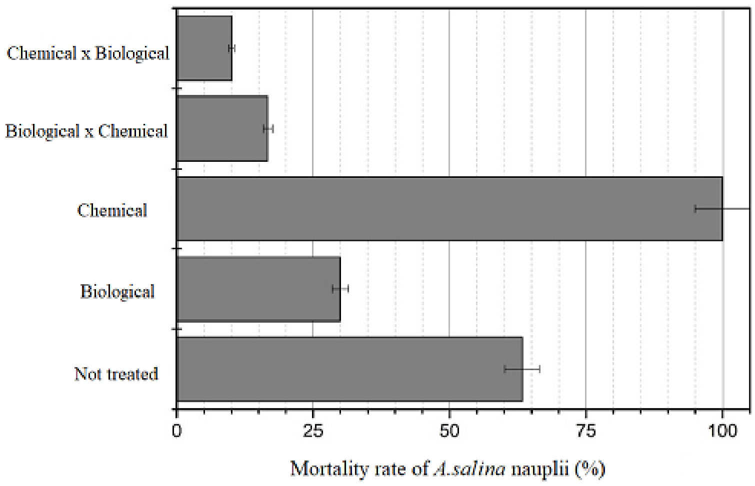

Figure 2. Comparison of mortality rates (graph).

Table 3 shows the efficiencies of other processes about effluent treatment from the wood laminating industry. As can be seen, the results obtained in this work for the parameters color and total phenols were superior to those of the Fenton type (Sotorriva *et al.*, 2008) and combined Biological (*P. ostreatus*)>>Chemical (photo-Fenton) (Heinz *et al.*, 2019), and the most efficient treatment reported by Aguiar *et al.* (2009) (biodegradation by *L. edodes*) required treatment times much longer than those practiced in this study.

Table 2. Remaining *A. Salina* Nauplii.

Analysis of Variance	Treatment
G.L waste	10
F treatments	24,27*
General Average	5,60
Standard deviation	1,32
DMS (5%)	3,54
CV (%)	23,51
Tukey test at 5%	
Not treated	3,67[a]
Biological	7,00[b]
Chemical	0,00[c]
Biol X Chem	8,33[a]
Chem X Biol	9,00[a]

Significance level: *:1%; GL: degrees of freedom; DMS: minimal significant difference; CV: Coefficient of variation; mean in columns followed by the same letter do not differ statistically (Tukey; $p \leq 0.05$).

Table 3. Performance of treatment processes for wood lamination wastewater treatment.

Process	Experimental Conditions	Treatment time	Removal Efficiency	Ref.
Foto-Fenton (Fe^{2+} / H2O2)	$[H_2O_2]$ = 3330 - 5650 mg.L^{-1} a 33% v/v, Ferrous Sulfate Heptahydrate (10.000 mg.L^{-1}) Fe^{2+} 470 – 810 mg.L^{-1} , natural light as radiation source Constant Stirred	100 minutes	96% BOD, 88% COD, 96% color units	Sottoriva *et al.* (2008)
Biotreatment using white root fungi, *Lentinula edodes* UEC-2019 in an orbital (*shaker*) and bench bioreactor	pH 5,5, *shaker* at 100 rpm, 37ºC, Addition of glucose (79 g / L) as co-substrate	30 days	99% COD, 97% (color units), 92% (total phenols)	Aguiar *et al.* (2009)
Combined fungal and photo-oxidative Fenton processes	1:1 or 1:5 M ratio (Fe^{2+}/H_2O_2), pH 3; Temp. 25ºC, mercury lamp as radiation source (125 W, 81,9 W/m^2 and λ = 254 nm) biological treatment with *P. ostreatus* EB 016 in an air-lift reactor	60 minutes 40 days	99, 2% COD; 92, 2% phenolic compounds.	Heinz *et al.* (2019)
Combination Catalytic Ozonation x Biological Process	$[O_3]$ = 1 LPM, pH 5, [Catalyst] = 0,35 /mL Fungal biomass (16 ± 0,1 ± g), effluent (400 mL) supplemented with 79 g.L^{-1} dextrose co-substrate, pH 5± 0,1, Temp. 28-30ºC,	120 min 21 days	96,2% (sol. Lignin) 99,9% (total phenols) 93,3% color units	This work

4. CONCLUSIONS

The polluting potential of the effluent confirmed the pattern reported in the literature, justifying the need for treatability studies to promote the adequacy of environmental relevance parameters, especially phenols, color, acute toxicity, and biodegradability. Among the evaluated treatment methods, it was found that the isolated processes did not produce the best reduction efficiencies of the evaluated indicators. On the other hand, the integrated processes of catalytic Ozonation and Fungal Bioremediation (chemical>>biological) reached high levels of efficiency, with emphasis on the reduction indices of phenolic compounds and acute toxicity.

Although complementary studies for the optimization of the process are necessary, the results of this study, together with the advantages of the proposed chemical process, are considered of low relative cost, mainly because it uses catalytic material based on tailings from the steel industry. The results also highlight the significant potential of this combinative treatment process to improve the quality and minimize the impact of effluents generated by the wood laminate industry.

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