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TREATMENT OF TANNERY DYE-CONTAINING EFFLUENTS USING A NATIVE FUNGAL STRAIN

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1. Introduction

Dyeing is an important step in the leather supply chain, where the sensory characteristics and colouration of the surface are imparted to the final product. This stage is carried out in the post-tanning operations and is performed in aqueous medium (wet finishing), where a high volume of wastewater-containing dyes is produced. Other different chemical compounds such as deacidulants, retanning agents, fatliquoring oils, surfactants, chemical auxiliaries and acids are also added to the wet finishing process to guarantee the desired physical-chemical properties of leather and good absorption and fixation of dyes (Piccin et al., 2016). The composition of the wastewater generated in the dyeing step is very variable and depends on various factors such as the tanning and dyeing technology applied, and the types of leather and dye selected (Püntener, 2000). Tannery wastewaters from wet-ending processes are characterized by high biological oxygen demand (BOD), high chemical oxygen demand (COD) and high concentrations of total organic carbon (TOC) and nitrogen (Gutterres et al., 2015). These kinds of wastewaters are usually treated by mechanical, physical-chemical and biological treatments; however, effluents from this stage are difficult to treat by conventional methods because of the presence of dyes (Gomes et al., 2016). Therefore, the treatment of dye-containing wastewaters from tanneries still remains a challenge that motivates the application of new and advanced environmentally friendly strategies such as mycoremediation. Mycoremediation refers to the use of fungi to biodegrade and biodeteriorate different organic compounds for the treatment of recalcitrant compounds, which leads to a decrease in the pollution parameters such as colour, COD, BOD, TOC and toxicity (Singh, 2006). The use of white-rot fungi (WRF) has been proven to be a suitable alternative for the treatment of dye-containing wastewaters from textile industry.

The potential of these fungi is related to their biodegradation ability, carried out by their highly oxidative, non-specific and non-stereoselective enzyme system (Ali, 2010). Other mechanisms such as biosorption and bioaccumulation could also be involved in dye removal by the fungal mycelia (Kaushik and Malik, 2009).

Most research on biodegradation/biodecolourization by fungi is focused on the treatment of individual dyes from aqueous solution or simulated textile wastewater (Rodriguez-Couto, 2013). Wastewaters from leather dyeing are different from those of the textile industry because of the presence of specific chemicals used in leather production. The fungal treatment of leather dyes and tannery wastewaters are scarcely reported (Anastasi et al., 2010; Baccar et al., 2011; Rodriguez-Couto et al., 2004). Furthermore, very few papers related with the decrease of other pollution parameters such as COD, BOD and TOC and the determination of toxicity after mycoremediation with WRF are found in the literature (Anastasi et al., 2010; 2012; Ma et al., 2014., Placido et al., 2016).

In our previous study, the strain Trametes villosa SCS-10 was collected, isolated and selected for its efficient decolourization ability (over 90%) against the leather dyes Acid red 357, Acid Blue 161 and Acid Black 210 in aqueous solution. The results showed that the mechanisms of enzymatic biodegradation and biosorption played an important role in the colour removal (Ortiz-Monsalve et al., 2017; Puchana et al., 2017).

The aim of the current study was to evaluate the ability of Trametes villosa SCS-10 in the biodecolourization and biodetoxification of wastewaters from leather dyeing, assessing two types of wastewater composition for two different dyes (Acid Red 357 and Acid Orange 142) and three conditions of nutrient source supplementation. The efficiency of the treatment was assessed in terms of biodecolourization, reduction of pollution parameters (COD, BOD and TOC) and biodetoxification. Laccase activity and biomass production were monitored during the experiment.

2. Materials and methods

2.1. *Strain and inoculum*

The fungus *Trametes villosa* SCS-10 was collected and selected in a previous study for its efficient decolourization ability against different leather dyes due to the mechanism of enzymatic biodegradation (Ortiz-Monsalve et al., 2017) and biosorption (Puchana et al., 2017). The strain is preserved at the Culture Collection Mycoteca URM (Federal University of Pernambuco, Mycology Department, Brazil) under the access number URM-7641. For inoculum preparation, the strain was grown on Malt Extract Agar (Merck, Germany) plates for 7 days at 30°C. Three agar plugs of growing mycelium (3 mm) were used to inoculate each flask.

2.2. *Dye-containing wastewaters*

Dye-containing wastewaters were produced in a laboratory-scale tannery drum (Mathis LFA model, Mathis AG, Switzerland) using a formulation (Table 1) that reproduces an industrial process of wet-finishing of wet-blue leather (chromium-tanned leather). The residual waters

generated in each step of the process were collected, and two different types of wastewater were composed. Composite wastewater W_1 (total wastewater composition) was obtained by mixing all residual water produced from the steps (1) to (7): soaking to final washing. Composite wastewater W_2 (partial wastewater composition) was formed only with the effluent from the following steps after the addition of dye: from the step (4) fatliquoring, retanning and dyeing to (7) final washing.

These two types of wastewater compositions were used to assess the effect of the dye concentration on the treatment: wastewater W_1 had a high volume of water of the steps (1) to (3) and dye was diluted, while in W_2 the concentration of dye was higher. Two different leather dyes were used individually: Acid Red 357 (AR_{357}) and Acid Orange 142 (AO_{142}). These dyes are commonly used in the industrial dyeing of leather (acid type, azo chromophore and metal complex dyes) and were provided by Lanxess (São Leopoldo, RS, Brazil). All wastewaters produced were stored at 10°C.

2.3. *Decolourization and enzyme assays*

Assays of decolourization were performed testing three different treatments related with conditions of carbon and nitrogen sources supplementation. In the first treatment, a condition of high nutrient source supply (N_1) was assessed. In this case, the composition of the optimal medium obtained in a previous study (Ortiz-Monsalve et al., 2017) was emulated: 2% (m/v) of malt extract and 1% (m/v) of glucose. The second treatment consisted of a reduced nutrient source supply condition ($N_{0.5}$), where the concentration of nutrients was reduced by 50% (1% of malt extract and 0.5% of glucose). These nutrients were aseptically added to the wastewaters. In the third treatment, a condition of no nutrient addition (N_0) was assessed. All wastewaters were inoculated as described earlier and incubated under submerged fermentation conditions (30°C and 200 rpm for 11 days). Samples were withdrawn daily from the liquid cultures, centrifuged and micro-filtrated. The supernatant was used to measure decolourization and laccase enzyme activity. Mycelia were collected at the end of the treatment and biomass production was determined gravimetrically, by drying at 105°C for 24 h until constant weight was obtained.

Decolourization was determined by UV-vis spectral analysis in a range between 400–800 nm and was expressed in terms of biodecolourization efficiency: $\text{BE}\ (\%) = \frac{A_o - A_t}{A_o} \times 100$, where A_o and A_t represent the initial and post-treatment absorbance, respectively, at the λ_{Max} of each dye in the wastewater.

Extracellular laccase activity (Lac) was determined using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid, Sigma-Aldrich, USA) as substrate (Wang and Ng, 2006). The reaction consisted of an aliquot of culture sample added to ABTS solution (1 mM). The change in absorbance was monitored at 405 nm ($\varepsilon 405 = 3.6 \times 104\ M^{-1}\ cm^{-1}$) for 5 min. One unit of Lac activity was defined as the amount of laccase that catalysed 1 μmol of ABTS per minute.

Activities were expressed as U L^{-1}. Manganese peroxidase activity (MnP) was determined as described by Arora and Gill (2001). Lignin peroxidase activity was measured as reported by Tien and Kirk (1984). Only Lac activity was found in the tested conditions.

Table 1 – Leather wet-end formulation: composition of the dye-containing wastewaters

Step	Compound	Composition (%)[b]
(1) Soaking	Water	200
	Formic acid	0.2
	Surfactant	0.2
(2) Deacidification	Water	200
	Sodium formate	1
	Sodium bicarbonate	0.5
	Neutralizing agent	1
(3) Washing	Water	200
(4) Fatliquoring, retanning and dyeing I	Water	100
	Retanning agent	8
	Vegetable tanning agent	4
	Dispersing agent	1
	Synthetic oil	6
	Formic acid	2
	Dye[a]	2
(5) Washing II	Water	200
(6) Dyeing II	Water	100
	Formic acid	1
	Dye[a]	1.5
(7) Final washing	Water	200

[a] Dyes AR_{357}(λ_{max}: 494 nm; CAS: 57674-14-3) and AO_{142}(λ_{max}: 480 nm; CAS: 55809-98-8) were used individually in this stage; [b] The percentage was based on the weight of a half-bovine hide (approx. 150 g).

2.4. *COD, BOD and TOC assays*

The wastewaters were analysed before and after the fungal treatment for the chemical oxygen demand (COD), biochemical oxygen demand (BOD), total organic carbon (TOC) and pH. The procedures were developed as outlined in the Standard Methods (APHA, 2005). COD was determined with the dichromate method using a thermoreactor (Eco 6 model, VELP Scientifica,

Italy). BOD was measured with the respirometric 5-day BOD method using a BOD Sensor System (BOD Sensor System 6 model, VELP Scientifica, Italy). TOC was assessed in a TOC/TN-L analyser (SSM- 5000A, Shimadzu, Japan). Table 2 shows the characterization of the raw wastewaters before treatment.

Table 2 – *Physico-chemical characterization of the dye-containing wastewaters*

Wastewater	Physico-chemical parameters					
	pH	Colour	$\lambda_{max\ (nm)}$[a]	COD (mg L^{-1}) [b]	BOD (mg L^{-1})[c]	TOC(mg L^{-1}) [d]
W_1-AR_{357}	4.44	Dark red	494	6906.05 ± 224.74	2816.66 ± 76.38	3477.00 ± 88.34
W_2-AR_{357}	4.27	Dark red	494	9519.19 ± 463.67	3141.66 ± 57.73	4870.67 ± 44.81
W_1-AO_{142}	4.59	Dark orange	484	6816.74 ± 287.07	2758.33 ± 76.38	3399.50 ± 71.50
W_2-AO_{142}	4.19	Dark Orange	484	9887.68 ± 281.43	3525.00 ± 50.00	4797.67 ± 56.22

[a] Wavelength of maximum absorbance; [b] Chemical oxygen demand; [c] Biochemical oxygen demand; [d] Total organic carbon.

2.5. *Ecotoxicity assays*

2.5.1. *Toxicity*

Ecotoxicological characterization of wastewaters was performed before and after fungal treatment. Toxicity was analysed by measuring the ability of the wastewater to inhibit the luminescence of the aquatic bacteria *Vibrio fischeri* and the growth of the green unicellular alga *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata/Selenastrum capricornutum*).

The test on *Vibrio fischeri* was carried out according to ISO 11348-3 (ISO, 2007) and the Brazilian method NBR 15411-3 (ABNT, 2012). Data were expressed as the effective concentration that provokes 50% of light reduction by the toxic effect (EC_{50}, %).

The algae growth inhibition test was set up according to the OECD 201 guidelines (OECD, 2011) and NBR 12648 (ABNT, 2011). The exposure time was 96 h. Data were expressed in terms of the effective concentration that inhibits the algal growth by 50% (IC_{50}, %).

2.5.2. *Cytotoxicity*

The cytotoxicity assays were conducted in a permanent lung fibroblast cell line derived from Chinese hamsters (V79). The cell line was cultivated under standard conditions in Dulbecco's

modified Eagle medium (GIBCO) supplemented with 10% heat-inactivated Foetal Bovine Serum (GIBCO), 0.2 mg/mL L-glutamine, 100 IU/mL penicillin and streptomycin, and 0.1% fungizone. Cells were preserved in tissue-culture flasks at 37°C in a humidified atmosphere with air containing 5% CO_2 and were harvested by 0.15% trypsin–0.08% EDTA in PBS. Before treatment, V79 cells (1×10^4 cells) were seeded in complete media and cultured for 24 hours in 96-well plates for MTT assays and the effluents were filtered with PES membranes with a pore size of 0.22 µm (syringe filters - K18-230, Kasvi, PR, Brazil). Untreated and treated wastewaters were added to complete media to achieve the different desired volume of wastewater (20, 40, 60, 80, and 100 µL in 200 µL of final volume), and cells were treated for 3 h and 24 h under standard conditions. The negative control was exposed to an equivalent concentration of solvent.

MTT (3- (4.5-dimethylthiazole-2-yl) −2.5-biphenyl tetrazolium bromide, Sigma-Aldrich, USA) reduction was performed according to Denizot and Lang (1986) with a few modifications (Jaramillo-García et al., 2018). In summary, at the end of the treatments, 20 µL of yellow tetrazolium salt (MTT; 4 mg/mL) per well was added and incubated in the dark for 3 h at 37°C. After incubation, the supernatant was carefully removed, the residual purple formazan product solubilized in 0.2 mL Dimethyl sulfoxide (Sigma-Aldrich, USA), stirred for 5 min and the absorbance measured at 540 nm with a microplate reader (EnSpire Multimode Plate Readers - PerkinElmer Inc.). The absorbance of negative control cells was set as 100% viability and the values of treated cells were calculated as percentage of control.

2.6. *Data analysis*

Data were expressed as the mean of triplicates with the standard error (± SE). Analysis of variance (ANOVA) was carried out using Statistica v10.0 software (StatSoft, USA). Differences among treatment means were considered significant when p was ≤ 0.05 by the Tukey-Kramer test.

3. Results and discussion

3.1. *Kinetics of biodecolourization of dye-containing wastewaters*

Trametes villosa SCS-10 showed strong resistance and versatility in the treatment of all the different kinds of wastewaters. The strain demonstrated a remarkable biodecolourization efficiency (BE, %) of the total composite wastewater (W_1), achieving between 85–95% of colour removal after 264 h of incubation (Table 3). The best values of biodecolourization were achieved

in the treatment with the reduced nutrient supply condition ($N_{0.5}$), with 93.76 ± 1.47% and 90.62 ± 0.67% for W_1-AR_{357} and W_1-AO_{142}, respectively. Although the treatment with the high nutrient source supply condition (N_1) also showed efficient colour removal, the biodecolourization was surprisingly lower: 89.96 ± 0.48% for W_1-AR_{357} and 84.52 ± 1.02% for W_1-AO_{142}. Previous results obtained in the decolourization of leather dyes from aqueous solution with *T. villosa* SCS-10 suggested that the treatment N_1 provided the best operational conditions (Ortiz-Monsalve, et al., 2017). However, this treatment (N_1) did not present the same efficiency of biodecolourization in the real wastewater. This shows the great influence of the composition of the nutrient sources in biodecolourization of real wastewaters. In addition, the treatment without nutrient supplementation (N_0) showed slight colour removal values, ranging between 50–70% for both wastewaters composites (W_1 and W_2) (Table 3). These data confirmed that the supplementation of nutrients is necessary to ensure efficient colour removal, since the biodecolourization is associated with the production of ligninolytic enzymes in secondary metabolism (Swamy and Ramsay., 1999). The results were less impressive for wastewaters W_2 wherein biodecolourization ranged between 75–90%, with the best performance also with the treatment $N_{0.5}$: 89.13 ± 0.63% and 89.61 ± 1.0% for W_2-AR_{357} and W_2-AO_{142}, respectively. This performance was related with the concentration of dyes: the W_2 wastewater composite had a high concentration of dyes, which may have adverse effects on biomass production, laccase activity and biodecolourization (Ali, 2010).

The kinetics of biodecolourization of the four wastewaters showed that the colour removal was strongly associated with the activity of the laccase enzyme (Fig. 1 a-b and Fig. 2 a-b). Overall, the efficient biodecolourization began when the activity of the laccase enzyme was higher. In the treatment $N_{0.5}$ of the W_1-AR_{357} and W_2-AR_{357}, for example, *T. villosa* SCS-10 reached the maximum rate of biodecolourization within 96–144 h, the period when laccase showed the highest levels (1000–1300 U L^{-1}) (Fig. 1a–d). The same behaviour was observed in the W_1-AO_{142} and W_2–AO_{142} wastewater samples (Fig. 2a–d). In the treatment N_1, the high rate of biodecolourization began 24–48 h later than with the $N_{0.5}$ treatment, between 144–168 h, which also coincided with the maximum peak of laccase activity and confirmed that a higher supply of nutrients can delay the biodecolourization/biodegradation of dyes.

These results showed similar trends to data obtained on the biodecolourization of leather dyes in aqueous solution, where the laccase inhibitor sodium azide was used to confirm that the colour removal was associated with the enzymatic biodegradation of dyes by the laccase enzyme (Ortiz-Monsalve et al., 2017).

The biodecolourization can also be correlated with bioaccumulation. The initial colour removal observed in the first 24–96 h of treatment was due to a mechanism of dye adsorption by the fungal growing mycelium (bioaccumulation). This mechanism is related with the production of biomass. The treatment with the higher supplementation of nutrients (N_1) showed a significant

difference in biomass production (see Table 3). Consequently, this condition proved high colour removal by bioaccumulation before laccase activity was detected. However, large biomass production appeared to have a negative effect on colour removal after laccase showed peaks of activity. The best performance of the reduced nutrient supply treatment may be associated with the fungal morphology. The treatment $N_{0.5}$ allowed mycelial growth in form of uniform pellets homogeneously distributed in the wastewater. In contrast, the high nutrient condition induced a heterogeneous mass of mycelia, with small flakes peeling away from a larger mass of mycelia. The pellet arrangement in the condition $N_{0.5}$ improved the mass transfer (oxygen and nutrients) from the liquid phase (culture medium) to the solid phase (growing cells) as reported by Kaushik and Malik (2009).

UV-vis spectrum analyses (400–800 nm) were performed after 264 h of treatment. In the W_1-AR_{357} and W_2-AR_{357} biodecolourization profiles, a decrease in the major peak present in the control (before treatment) wastewater at 494 nm was observed. In wastewater W_1 a complete decline in this characteristic peak in treatments N_1 and $N_{0.5}$ was observed. Treatment N_0 showed a slight decrease in the absorbance at 494 nm (Fig. 1e). In the partial composite wastewater W_2, the absorbance at 494 nm was significantly higher due to the high concentration of dye. The disappearance of the characteristic peak was only observed in the treatment $N_{0.5}$, while in N_1 and N_0, a decrease in absorbance related with the low biodecolourization was observed (Fig. 1f). In W_1-AO_{148} and W_2-AO_{148}, a unique peak at 484 nm was also observed. In treatment $N_{0.5}$, the peak disappeared almost completely for both composite wastewaters (W_1 and W_2) (Fig. 2 e-f). Abiotic controls did not show any change over the course of the treatment.

Table 3 – Biodecolourization, laccase activity, biomass production and mycoremediation (COD, BOD, and TOC removal) after treatment of dye-containing wastewaters by *Trametes villosa* SCS-10

Wastewater composition	Nutrient Condition	Colour removal			Physico-chemical parameters			
		BE (%)[a]	Lac (U L^{-1})[b]	Biomass (g L^{-1})	COD (mg L^{-1})[c]	BOD (mg L^{-1})[d]	TOC (mg L^{-1})[e]	pH
W_1-AR_{357}	N_0	69.35 ± 1.85	807.04 ± 30.17	1.11 ± 0.09	3817.19 ± 317.64	1758.33 ± 125.83	1256.00 ± 39.61	5.22 ± 0.07
	N_1	89.96 ± 0.48	1063.89 ± 33.68	2.73 ± 0.07	3930.51 ± 281.43	1666.66 ± 76.38	1564.67 ± 71.12	5.77 ± 0.12
	$N_{0.5}$	93.76 ± 1.47	1251.41 ± 29.05	1.49 ± 0.12	1805.94 ± 180.59	933.33 ± 57.73	647.3 ± 69.1	5.56 ± 0.08
W_2- AR_{357}	N_0	54.55 ± 1.07	520.74 ± 26.79	0.07 ± 0.02	4870.05 ± 209.57	2075.01 ± 132.29	2133.00 ± 48.87	4.40 ± 0.36
	N_1	79.78 ± 1.78	593.70 ± 51.46	2.08 ± 0.23	5598.42 ± 361.19	2058.33 ± 76.38	2703.01 ± 14.17	4.81 ± 0.14
	$N_{0.5}$	89.12 ± 0.63	806.30 ± 40.13	1.25 ± 0.15	3585.50 ± 147.04	1425.00 ± 86.60	1115.33 ± 75.79	4.58 ± 0.07
W_1-AO_{142}	N_0	65.75 ± 3.80	639.26 ± 41.82	1.09 ± 0.03	3830.40 ± 182.40	1741.66 ± 76.38	1322.00 ± 72.63	5.39 ± 0.11
	N_1	84.53 ± 1.02	755.18 ± 21.27	2.78 ± 0.10	3807.68 ± 562.87	1750.01 ± 50.02	1662.67 ± 61.08	5.65 ± 0.05
	$N_{0.5}$	90.62 ± 0.67	1040.74 ± 40.39	1.29 ± 0.02	2246.31 ±179.70	966.66 ± 28.87	599.50 ± 71.50	5.49 ± 0.01
W_2-AO_{142}	N_0	47.37 ± 3.49	516.41 ± 38.24	0.08 ± 0.02	5897.60 ± 278.62	2341.66 ± 125.83	2271.33 ± 62.74	4.29 ± 0.05
	N_1	74.32 ± 2.46	548.10 ± 26.29	1.97 ± 0.11	6320.79 ± 651.14	2458.33 ± 76.38	2626.67 ± 128.94	4.92 ± 0.13
	$N_{0.5}$	89.63 ± 1.00	773.70 ± 35.56	1.09 ± 0.05	3891.20 ± 379.70	1558.33 ± 125.83	1148.67 ± 49.08	4.66 ± 0.22

[a] Biodecolourization efficiency after 11 days of treatment; [b] Laccase activity peak during treatment; [C] Chemical oxygen demand; [d] Biochemical oxygen demand and [e] Total organic carbon after fungal treatment.

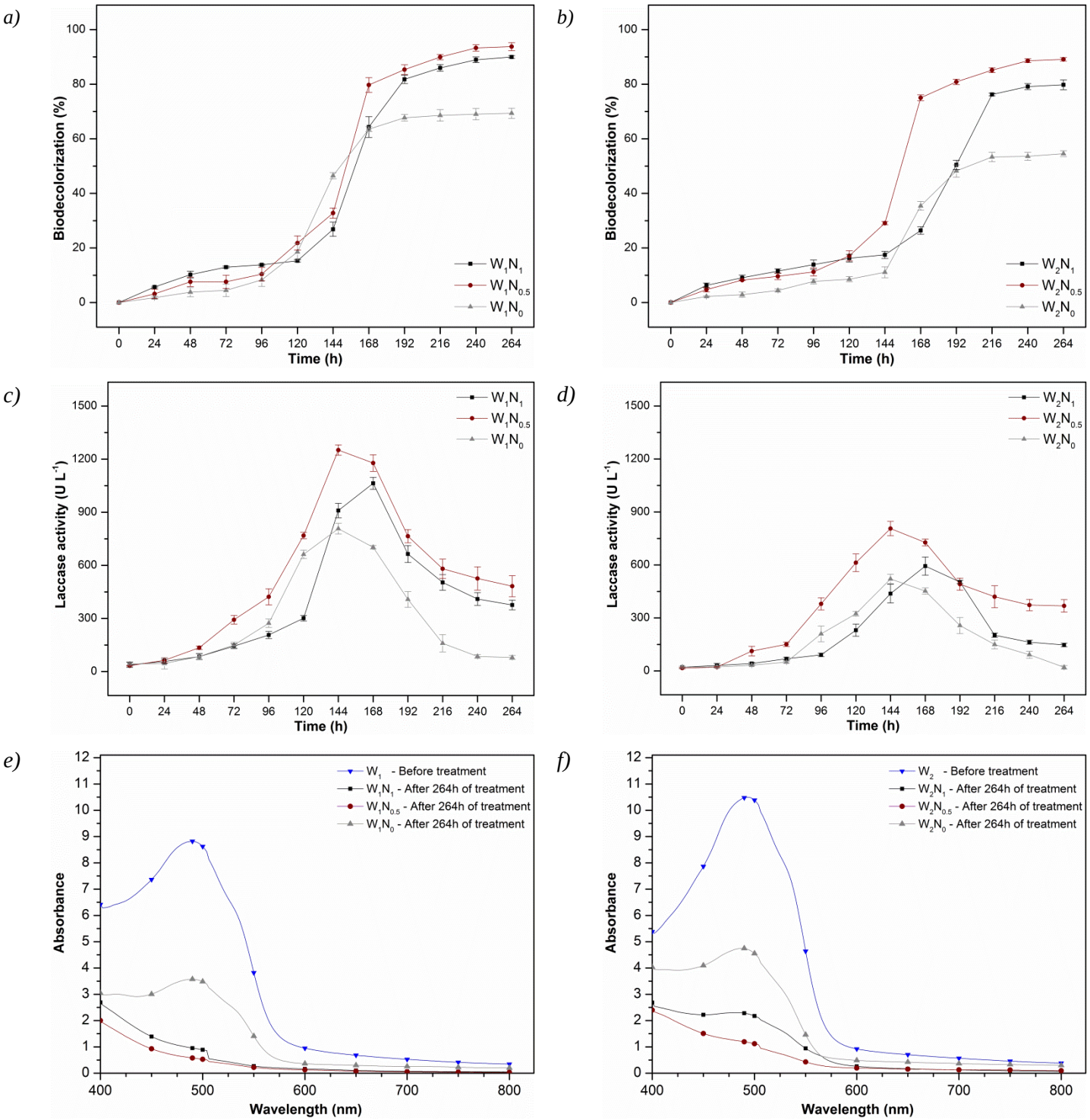

Figure 1 – Decolourization kinetics of leather dyeing wastewaters containing Acid Red 357 (WS-AR_{357}) by a native strain of *Trametes villosa* SCS-10: *a)* W_1 wastewater sample; *b)* W_2 wastewater sample. Laccase activity during the process of decolourization of wastewaters: *c)* W_1; *d)* W_2. UV-Visible spectrum analysis during biodecolourization: *e)* W_1; *f)* W_2. The bars indicate the standard error between three replications.

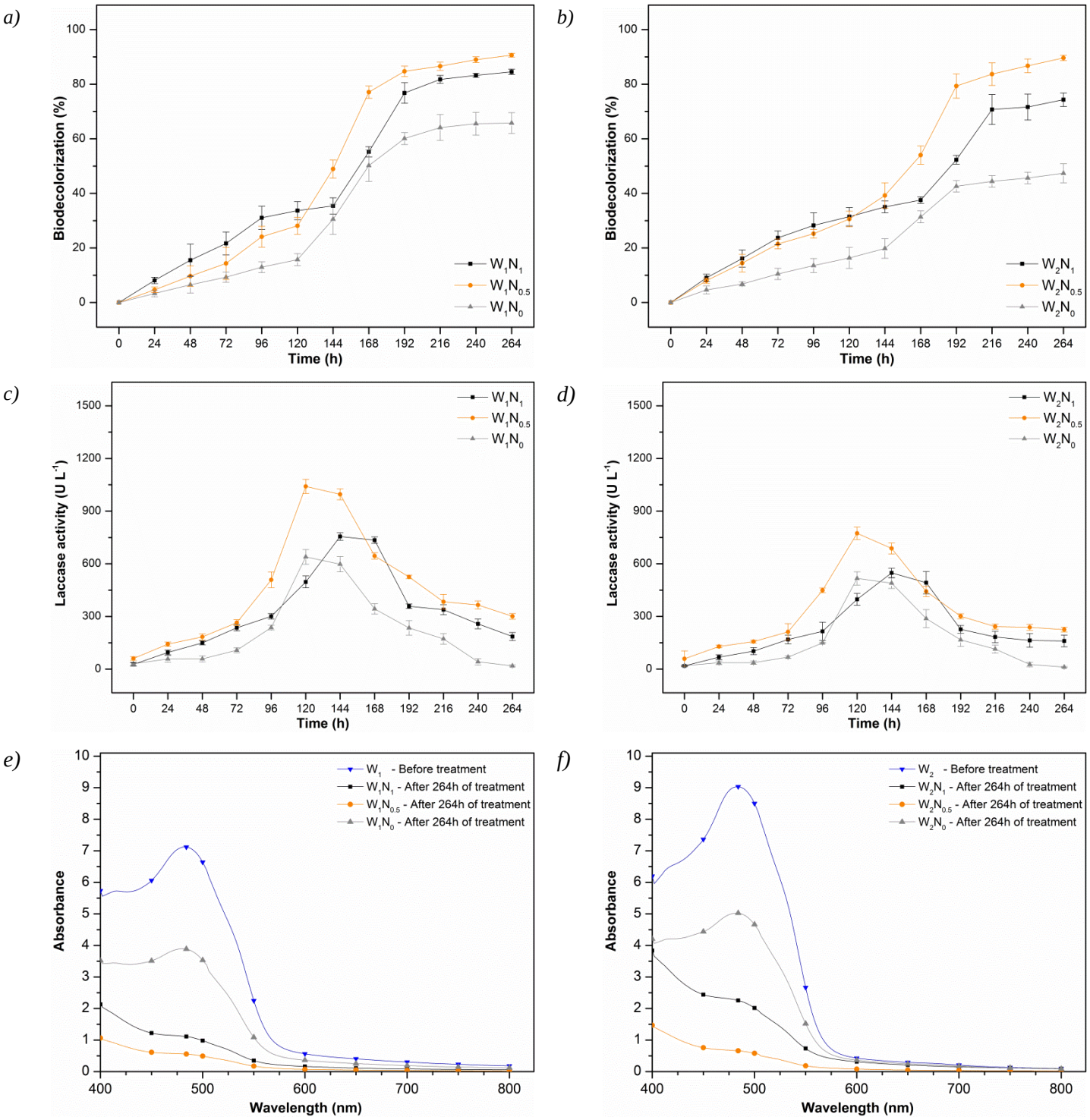

Figure 2 – Biodecolourization kinetics of leather dyeing wastewaters containing Acid Orange 142 (WS-AO_{142}) by a native strain of *Trametes villosa* SCS-10: *a)* W_1 wastewater sample; *b)* W_2 wastewater sample. Laccase activity during the process of decolourization of wastewaters: *c)* W_1; *d)* W_2. UV-Visible spectrum analysis during biodecolourization: *e)* W_1; *f)* W_2. The bars indicate the standard error between three replications.

3.2. *Mycoremediation: COD, BOD, and TOC decrease*

A decline in the COD, BOD and TOC values was observed after the fungal treatment with *T. villosa* SCS-10. Although biodecolourization is also a process of mycoremediation (Singh, 2006), in the current paper this term was used to refer to the decrease in COD, BOD and TOC. It is important to highlight that we observed an increase in each parameter due to the nutrient supplementation before the fungal treatment; this has not been considered for the final total removal. Figure 3 summarizes the mycoremediation performance of *T. villosa* on the wastewaters.

- *Chemical oxygen demand*: COD decrease varied significantly, ranging between 40–80% (Figure 3). Similar to the biodecolourization results, COD decrease was more efficient for the total composite wastewater (W_1) in the reduced nutrient supply condition ($N_{0.5}$): in the W_2-AR_{357}, for example, the COD was reduced from 6906.05 ± 224.74 to 1805.9 ± 180.6 mg L^{-1}(Table 3), representing approximately 73.85 ± 3.14% of COD decrease (Figure 3). COD represents the concentration of oxygen required to oxidize all carbon compounds susceptible to oxidation by strong chemical oxidants. The highest initial COD of the partial composition (W_2) before treatment (between 9500 and 1000 mg L^{-1}) was due to the high concentration of dye and other chemicals used in the wet ending formulation such as surfactants, oils and retanning agents. In the total wastewater composition (W_1), the dye concentration was lower, and consequently, the treatment allowed higher values of COD diminution. Otherwise, the reduced nutrient condition ($N_{0.5}$) also proved to be significantly better that the high nutrient condition (N_1) for COD removal. The higher nutrient condition resulted in COD removal values lower than 45% in all compositions (W_1 and W_2) for both dyes. Assuming the fungus did not consume all organic matter added as nutrient, the higher supply of nutrients may have affected the value of the organic load after treatment. A similar behaviour in COD after fungal treatment and culture medium supplementation was reported by Anastasi et al. (2012). The authors reached an efficient biodecolourization with fungal treatment and recommended an additional treatment, such as activated sludge, to achieve a better COD removal performance. Similar to the decolourization process, the supplementation of nutrients is necessary for the reduction of COD: the condition of non-addition of nutrients (N_0) barely allowed the removal of 40–50% of COD. The decrease in COD during the fungal treatment was due to the enzymatic degradation of complex compounds present in wastewater, such as dyes. COD removal values were comparable to those obtained by other authors for textile effluents or textile dyes in aqueous solution: Ma et al. (2014) used a strain of *Ganoderma* sp. for the treatment of simulated textile wastewater containing the dye Reactive Orange 16, achieving 61.6% of COD removal in 10 days. Sanghi et al. (2011) reported the reduction of 60 ± 10% of COD in the fungal treatment of 5 different textile dyes using the fungus *Coriolus versicolor*. It should be noted that COD values in wastewaters from textiles industries

are lower than those from tanneries, and data on the reduction of COD of dye-containing wastewater from tanneries using fungi were not found in the literature. The efficiency of the fungal treatment in terms of COD reduction was similar with other treatments of dye-containing wastewater from tanneries such as biological treatment, Fenton oxidation, adsorption and ozonation (Mandal et al., 2010, Mella et al., 2017, Preethi et al., 2009).

- *Biochemical oxygen demand*: BOD represents the quantity of oxygen required to biologically stabilize the organic matter in wastewaters. The treatment with *T. villosa* SCS-10 allowed 64.95 ± 0.71 and 66.86 ± 1.22% of BOD removal in the nutrient condition $N_{0.5}$ for W_1-AO_{142} and W_1-AR_{357}, respectively. No studies were found in the literature dealing with the decrease in BOD from tannery wastewater using fungal treatment. Few previous reports on biotreatment of dye-containing wastewaters from textile industry measuring BOD removal were found: Asgher et al. (2014) observed a reduction of 80–96% in BOD during the treatment of textile wastewaters with enzyme extracts of *S. commune*.

- *Total organic carbon*: above 80% of reduction of TOC was observed in treatment $N_{0.5}$ for both effluents W_1-AR_{357} and W_1-AO_{142}. The fungal treatment allowed a TOC reduction from 3477.00 ± 88.34 to 647.33 ± 69.14 mg L^{-1} (Table 3) in the W_1-AR_{357} wastewater sample, representing 81.38 ± 1.50 of TOC removal. A similar performance was observed in the W_1-AO_{142} wastewater with 82.37± 1.73 of TOC removal (Figure 3). This efficiency in the reduction of total organic carbon suggests the presence of disrupting reactions of dye molecules and other chemical compounds present in wastewater, resulting in simpler fragments. Similar results were reported by Asgher et al. (2014) in the treatment of textile effluents by crude ligninolytic enzymes extract from *Schyzohyllum commune* IBL-06. Rosales et al. (2011) also found a reduction in TOC when comparing the efficiencies of the decolourization of leather dyes by enzymatic and electrochemical treatments. However, the authors reported just 23% of TOC reduction by the commercial laccase from *Trametes versicolor*. Similarly, Novotný et al. (2011) studied the colour removal and TOC reduction of dyes from aqueous solution based on the enzymatic degradation by fungus *Irpex lacteus* followed by anaerobic degradation in bacterial reactors. Authors reported TOC reduction with fungi treatment; however, the second step using bacteria was more efficient in TOC removal. According to the literature, an additional treatment is necessary to complete the TOC removal from wastewater, since fungi can initiate the degradation of recalcitrant compounds but are not able to achieve complete mineralization (Hai et al. 2008). Therefore, the treatment of dye containing wastewaters may be enhanced using microbial consortia consisting of both fungi and bacteria to achieve efficient biodegradation and mineralization of synthetic dyes and other organic xenobiotics (Ali 2010).

- *pH*: pH was measured in the untreated and fungi-treated wastewaters. pH values ranged between 4.0–6.0 during the fungal treatment with *T. villosa* SCS-10 (Table 3). Overall, the final pH of wastewater W_1 in both treatments N_1 and $N_{0.5}$ varied between 5.4–5.8. This pH value is close of 5.5, the optimum for laccase activity of *T. villosa* SCS-10 according to Ortiz-Monsalve et al. (2017). This could be a factor for the slightly higher values of efficiency of biodecolourization and Lac activity of the W_1 wastewaters. Many authors report that fungi show better biodecolourization efficiency at acidic or neutral pH (Ali, 2010; Kaushik and Malik, 2009). The acidic conditions of the W_2 wastewaters also allowed high values of Lac activity and colour removal. Previously, *T. villosa* SCS-10 showed high biodecolourization and Lac activity in a wide pH range (4.0–8.0) (Ortiz-Monsalve et al. 2017). A similar behaviour was reported in *Trametes trogii*, which was efficient in the treatment of the textile dye Astrazon Red FBL at pH 6.0–11 (Yesilada et al. 2002). Although acidic or alkaline condition have been reported as inhibitory to the treatment of wastewaters by white rot-fungi, *T. villosa* SCS-10 displayed resistance and versatility at different pH conditions during the treatment of the four wastewaters assessed. This is very important for the treatment of industrial wastewaters, since different pH conditions are found in tannery wastewaters.

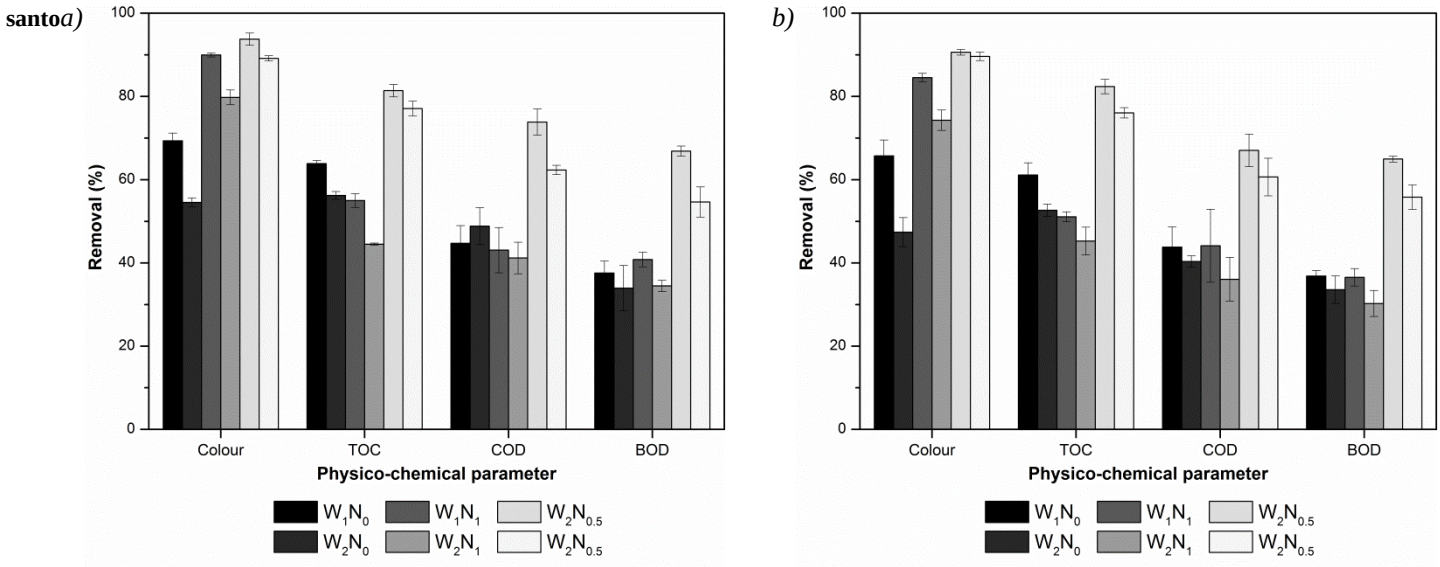

Figure 3. Biodecolourization efficiency and mycoremediation after 11 days of treatment with *T. villosa* SCS-10 of wastewaters W_1 and W_2 in the nutrient conditions N_1 and $N_{0.5}$ for: *a)* Wastewaters containing Acid Red 357 (W-AR_{357}) and *b)* Wastewaters containing Acid Orange 142 (W-AO_{142}).

3.3. *Biodetoxification*

The ecotoxicity characterization of the dye-containing wastewaters was carried out before and after the fungal treatment. *Vibrio fisheri* and *Raphidocelis subcapitata* were used to assess the toxicity. The total composite wastewater (W_1) was selected for the assay since colour removal and mycoremediation were more efficient in this composition. The results showed high toxicity in the raw effluent (Table 4), due to the chemicals added during the wet finishing process.

The test with *V. fisheri* showed that the toxic concentration EC_{50} of the raw wastewater W_1-AR_{357} was 0.48% in 30 min of exposure. However, the EC_{50} in the treated wastewater was 1.384 and 1.395% for the culture condition N_1 and $N_{0.5}$, respectively. The treatment $N_{0.5}$ allowed 65.53% of biodetoxification of the wastewater containing Acid Red 357. A similar result was observed in the wastewater W_1-AO_{142}, where the fungal treatment achieved 59.44% of biodetoxification. In the conditions assessed, there was not a significant difference in the reduction of toxicity for treatments N_1 and $N_{0.5}$. These data suggest that *T. villosa* SCS-10 is effective in reducing the toxicity of the wastewater. On the other hand, the test with *R. subcapitata* showed a slight reduction in toxicity. The IC_{50} of the untreated wastewaters were 0.60 and 0.46% for W_1-AR_{357} and W_1-AO_{142}, respectively. After the fungal treatment, IC_{50} was fluctuating between 1.30 and 1.60%, representing 60–70% of biodetoxification. Previously, Ma et al., (2014) observed the ability of *Ganoderma* sp. En3 to decolourize and detoxify textile wastewater containing Reactive Orange 16. Similarly, Anastasi et al., (2012) reported the biodetoxification capacity of *Trametes pubescens* on dye-containing wastewaters. However, the same authors described an increase of toxicity associated with the fungal treatment with *Bjerkandera adusta* and *Porostereum spadiceum.* Few reports dealing with the detoxification after fungal treatment were found in the literature (Anastasi et al., 2012; Chen et al., 2017; Khlifi et al., 2010), and in some cases an increase in the toxicity was found (Anastasi et al., 2011; Anastasi et al., 2012).

Although the ecotoxicity assays presented different results depending on the organism used and no treatment achieved a complete biodetoxification, the enzymatic degradation of the chemical compounds present in the effluents did not result in an increase in the toxicity. These results are very important to demonstrate the applicability of the fungal treatment. It is also important to highlight that the values of toxicity achieved for the treated wastewaters are within the Brazilian legal limit. Further studies are required to analyse the toxicity of the metabolites produced in the fungal biodegradation and to optimize the biodetoxification process. Others interesting strategies such as the use of two-steps process with fungi/bacteria (Anastasi et al., 2012; Novotny et al., 2011) or fungi/ozonisation (Vanhulle et al., 2008) to reduce the toxicity have been described by other authors.

Table 4 – Biodetoxification assays

Wastewater Sample/Toxicity assay	*Vibrio fisheri*			*Raphidocelis subcapitata*		
	EC_{50} (%)[a] Before fungal treatment	EC_{50} (%) After fungal treatment		IC_{50} (%)[b] Before fungal treatment	IC_{50} (%) After fungal treatment	
		Culture Condition N_1	Culture Condition $N_{0.5}$		Culture Condition N_1	Culture Condition $N_{0.5}$
W_1-AR_{357}	0.480	1.384	1.395	0.60	1.35	1.45
W_1-AO_{142}	0.568	1.327	1.401	0.46	1.59	1.47

[a] Effective concentration that provokes 50% of light reduction in *V. fisheri* by the toxic effect.

[b] Effective concentration that inhibits algal growth (*R. subcapitata*) by 50%.

Additionally, the MTT assay was used to evaluate cytotoxic properties of the untreated and treated dye-containing wastewaters. Due to the limited volume of wastewater, the test was carried out with the wastewater W_1-AR_{357}, which showed the best results for colour removal and mycoremediation. The cytotoxicity results are shown in Fig. 4. Compared to the negative control, a significant decrease ($p < 0.001$) was observed in the survival of V79 cells, induced by untreated wastewater (20 µL), in a dose-dependent manner after 3 h (Fig. 4a) and 24 h (Fig. 4b) of treatment.. On the other hand, treated wastewaters W_1-AR_{357} did not decrease the cellular viability when compared to the negative control. These results provide evidence that the treatment with *T. villosa* SCS-10 did not produce toxic compounds that affected the viability of V79 cell line and agreed with the previous ecotoxicity assays. These results are a crucial confirmation that the biodecolourization and mycoremediation of the wastewaters did not produce cytotoxic metabolites and is a viable treatment for real-world applications in tanneries. Studies analysing the reduction of cytotoxicity in tannery effluents using fungi were not found in the literature, however our results are comparable to similar assays carried out in the fungal treatment of textile wastewaters: Placido et al. (2016) demonstrated how the WRF *Leptosphaerulina* sp. did not produce toxic metabolites as tested on the human cell line U937. Similarly, the MTT assay on the Caco-2 cell line found no toxicity associated with WRF *P. sanguineus*-treated dye-containing wastewater (Vanhulle et al. (2008).

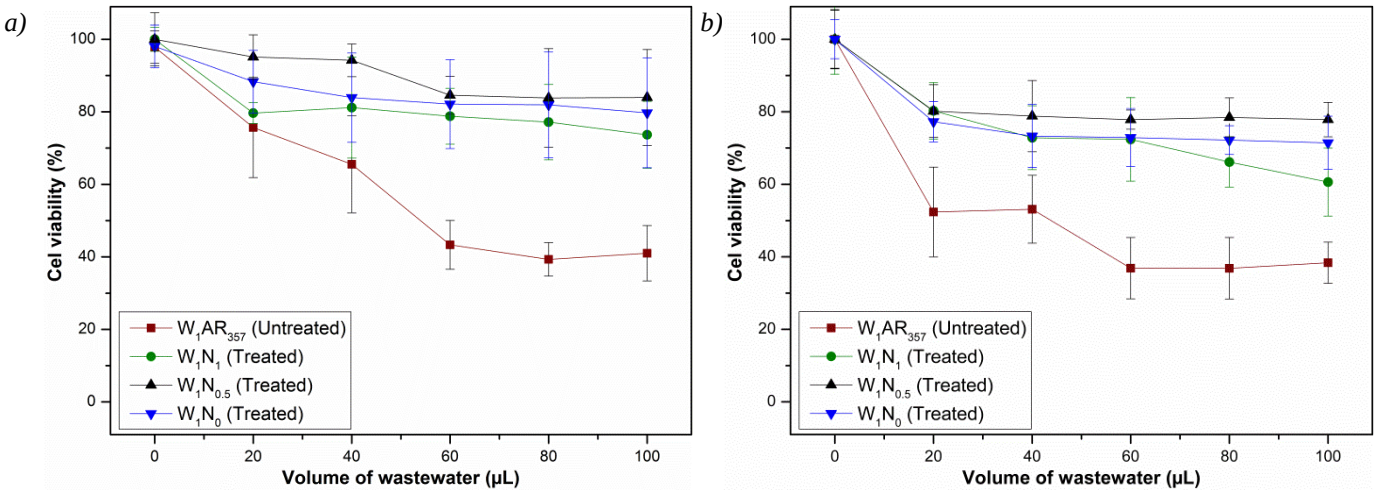

Figure 4. Cell viability of the cell line V79 after 11 days of fungal treatment (*T. villosa* SCS-10) of the wastewater W_1-AR_{357} in the nutrient conditions N_1,$N_{0.5}$ and N_0 for: *a)* cytotoxicity test of 3 h and *b)* cytotoxicity test of 24 h.

4. Conclusions

Trametes villosa SCS-10 showed versatility and resistance in the treatment of four dye-containing wastewaters. The fungal treatment was efficient, achieving colour removal of over 90% (biodecolourization) and COD and TOC removal of over 80% (mycoremediation). The best results were achieved when wastewaters were supplemented with a reduced nutrient source supply (treatment $N_{0.5}$) due to the better physiological conditions that allowed for the highest peaks of laccase activity (1000–1300 U L^{-1}) and the pellet arrangement of the mycelia. The treatment also reduced the ecotoxicity by 50–70% and did not present cytotoxic effects to the cell line V79. *T. villosa* SCS-10 is considered a suitable candidate for the treatment of dye-polluted wastewater from leather dyeing. Further studies are required to improve the applicability of selected fungal strains in the treatment of wastewater from the tannery industry.

5. Suggestion for Future Work

The promising results in this project allow the field of research to be open to other possibilities, focused primarily on the scaling up and real application in tanneries. One of the objectives of the project for the current year is to evaluate the operation in different reactor configurations up (batch, air fluidized, expanded-bed or fixed-bed bioreactors), increasing the scale of process. The application on a large scale also requires the study of the immobilization of cells and enzymatic extracts, which is currently being assessed in LACOURO. Is also necessary, considering the possible industrial application, to study the use of economic substrates and sources of nutrients, mainly waste materials from other industries such as lignocellulosic materials from paper and wood industry. Further studies are also required to characterize the metabolites produced in the treatment and the pathways of degradation.

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PPGEQ
PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA QUÍMICA

Review to the Project:
TREATMENT OF TANNERY DYE-CONTAINING EFFLUENTS USING A NATIVE FUNGAL STRAIN

Santiago Ortiz-Monsalve – YLSG 2017

In the project the native fungal strain *Trametes villosa* SCS-10 was investigated for its ability to treat real wastewaters from leather dyeing produced in a laboratory-scale tannery drum. Two types of wastewater composition using two different dyes (Acid Red 357 and Acid Orange 142) were produced and used in the assays. Three culture conditions were studied in order to improve the yield of the treatment. The efficiency of the treatment was assessed in terms of biodecolourization, mycoremediation (reduction of pollution parameters: COD, BOD and TOC) and biodetoxification. Laccase activity and biomass production were monitored during the experiment. The fungal treatment resulted in between 50–70% of colour removal and 40–60% of COD and TOC removal of the raw undiluted wastewater without carbon and nitrogen supplementation (N_0). However, when wastewaters were supplemented with a high and a reduced nutrient supply (conditions N_1 and $N_{0.5}$, respectively), higher values of biodecolourization (over 90%) and COD and TOC removal (over 80%) were achieved. Experiments with the reduced nutrient source supplementation allowed the best physiological conditions for colour, TOC and COD removal, which were related to the highest peaks of laccase activity (1000–1300 U L^{-1}), the pellet arrangement of the mycelia and the lowest biomass production. BOD removal was less impressive during the treatment. The ecotoxicity assays with *R. subcapitata* and *V. fisheri*demonstrated that the fungal treatment resulted in 50–70% of biodetoxification of W_1-AO_{142} and W_1-AR_{357}. The MTT assay revealed that the metabolites produced during the treatment of W_1-AO_{142} wastewater did not present cytotoxic effects to the cell line V79. These promising results show that *T. villosa SCS-10* is suitable for the treatment of wastewater from leather dyeing. Further studies are required to improve the applicability of selected fungal strains in the treatment of wastewater from the tannery industry.

The major findings are resumed:
- The native fungi *T. villosa* SCS-10 was used in the treatment of real wastewaters from leather dyeing, produced in laboratory-scale tannery drum.
- *T. villosa* allowed the biodecolourization of the wastewater, achieving over 90% of colour removal.
- The mycoremediation showed high efficiency, resulted in COD and TOC removal of over 80%.
- The treatment of the wastewater reduced toxic effects over *R. subcapitata* and *V. fisheri*
- The fungi-treated wastewaters did not present cytotoxicity in the cell line V79

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