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MICROBIOLOGY

Biotechnological potential of microorganisms from textile effluent: isolation, enzymatic activity and dye discoloration

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Abstract: Environmental pollution may be considered one of the main problems affecting the world population. As the effluents from textile industries are the largest representatives of sources of pollution of water bodies due to the disposal of colored compounds in the environment. Microorganisms capable of thriving in textile wastewater may exhibit metabolic machinery to synthesize a wide variety of enzymes and/or secondary metabolites of industrial interest. The present work investigated the biotechnological potential of filamentous fungi from wastewater of a textile industry for the production of laccase, cellulase, amylase and lipase enzymes and their potential for discoloration capacity of Remazol Brilliant Blue R synthetic dye. The isolate *Aspergillus sydowii* (ITF 30) presented the best cellulase (46.74 U mL^{-1}), amylase, lipase and laccase (0.0273 U L^{-1}) production, as well as RBBR dye discoloration ability in solid medium, followed by isolate *Aspergillus sydowii* (ITF 27) able to synthesize cellulase, amylase and laccase and had the capacity to discolor 74.7% of RBBR in liquid medium. The results of the present work encourage future studies of characterization, optimization and purification of the enzymes encountered, aiming to be used in bioremediation processes of textile industrial effluents.

Key words: Amylase, bioremediation, cellulose, laccase, lipase, RBBR.

INTRODUCTION

With the growth of the world population, the demand for products increases proportionally, resulting in a greater amount of industrial waste. Large volumes of waste are incorrectly disposed in the environment, consequently causing high impact pollution (Hoornweg et al. 2013, Ghaly et al. 2014).

Textile industry can be considered one of the major industries in the world and play a major role in the economy of many countries (Ghaly et al. 2014). Wastewater from the textile industry has a high amount of hazardous toxic chemical pollutants including sulphur, synthetic dyes such a Remazol Brilliant Blue R (RBBR), nitrates, acetic acid, soaps, chromium compounds, formaldehyde, hydrocarbon and heavy metals like copper, arsenic, lead, cadmium, mercury, nickel, and cobalt, making the effluent highly toxic and harmful to human and all life forms inhabiting the environment (Kant 2012, Ghaly et al. 2014). A significant amount of residual azo dyes from inappropriate discharge in the aqueous ecosystems leads to the reduction in biochemical oxygen demand (BOD) and chemical oxygen demand (COD), decreasing the water quality and transforming these water bodies a lethal environment to the resident microbial communities resident (Olukanni et al. 2006, Moreira-Neto et al. 2013, Zhezhova et al. 2014). There is a clear need to treat industrial

effluents in order to minimize their impacts and, in this sense, the application of sustainable technologies is a promising strategy.

The toxicity of compounds released by the textile industry directly interferes with the life of fish and others aquatic organisms such as plants, bacteria and fungi. Biological treatment of wastewater derived from textile industry has been widely used, and it is based on decontamination by the use of microorganisms as recyclers, which convert toxic organic compounds into simpler non-toxic products (Ferrera & Sanchez 2016).

Microorganisms that thrived in this environment have developed the ability to process these compounds. Xenobiotic compounds that do not belong to the main metabolism of microorganisms are processed and transformed into metabolically assimilable forms. Biodegradation of these compounds by microbial communities can generate a sharing network of organic nutrient sources, making the environment viable for the development of related organisms (Prabha et al. 2017). The accumulation of the chemical charges in the ecosystem over time leads to the adaptation of microbial communities, depending on the type of chemicals present. The difference in microbial composition as well as its density in polluted and unpolluted waters is closely related to the amount of discharged chemical pollutants (Meerbergen et al. 2016).

Microbial community from wastewater treatment has been studied. Khan et al. (2013), performed a work using FISH (*Fluorescence In Situ Hybridization*) to monitor the bacterial microbial community in the activated sludge system of municipal wastewater treatment plant in Dubai. The authors found members belonging to archaea domain and bacteria. Faryal & Hameed (2005) sampled effluent and stream water from textile industry in Rawalpindi, Pakistan and verified that species from genera *Penicillium, Rhizopus* and *Candida* were the three most abundant fungal genera.

The use of microbial communities recovered from textile industrial effluents represents a promising strategy, considering the metabolic arsenal of these microorganisms with potential for application as environmental bioremediators of xenobiotic compounds due to the genetic and biochemical adaptation of this community to the toxic compounds found in the resulting effluents from these processes. In this way, the present work evaluated the production of lipase, amylase, cellulase and laccase enzymes as well as the discoloration of the synthetic dye RBBR, using filamentous fungi isolated from samples of a textile wastewater treatment plant for future bioremediation studies of industrial effluents.

MATERIALS AND METHODS

Sample collection and isolation

One sample was obtained from a wastewater treatment plant of a textile industry located in the city of São Miguel do Iguaçu, in western Paraná State (latitude -25.35424542 and longitude -54.24294591). The sample was collected using a sterile 500 mL glass vial and was subjected to pH and temperature analysis at the time of collection, using pH tape and thermometer, respectively.

The sample was processed by the serial dilution method (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) and aliquots of 50 µL from each sample dilution were used for inoculation in culture media for isolation of filamentous fungi. Fungal isolation was performed by inoculating the dilutions in Potato Dextrose Agar – (PDA) (10 g L^{-1} of glucose, 15 g L^{-1} agar in 1000 mL potato infusion, added of 500 mg L^{-1} of amoxicillin). The plates were kept at 28 °C for 30 days for the growth of filamentous

fungi. The isolates were preserved at -20 °C using 20% glycerol.

Morphological identification of fungal isolates

Morphological identification of fungal isolates was performed through macro and microscopic analysis. Initially, the strains were cultivated in PDA culture medium for 7 days at 28 °C. Colony colors and growth rates were evaluated with the aid of a stereoscope (NIKON SMZ 745 Model C -LEDS - China). The presence and size of sclerotia and conidia morphology were evaluated by the staining method on lactophenol slides with the aid of an optical microscope (NIKON Eclipse E200MVR - China).

Enzymatic Screening

Ligninolytic

For the evaluation of ligninolytic enzymes production, the filamentous fungi were cultivated on PDA as previously described, with the addition of 425 µl L^{-1} guaiacol 99% (indicator for phenol oxidases). For filamentous fungi, discs of 0.5 cm in diameter, obtained from the margins of the colonies, were inoculated in PDA plates for 7 days at 28 °C. Production of a brownish color around fungal mycelia was considered positive for ligninolytic activity and, the isolates ware submitted to enzymatic activity assay for the laccase enzyme (Desai et al. 2011). Experiments were performed in triplicate.

Lipase

Lipase activity was identified with the growth of microbial isolates in specific culture media. The filamentous fungi isolates were grown in RO-PDA culture media, according to Kumar et al. (2012). A solution (RO) of 31.25 mL of olive oil and 10 mL of Rhodamine B (1.0 mg mL^{-1}) was added with vigorous stirring. Discs of 0.5 cm in diameter were inoculated in RO-PDA plates. Plates were incubated for 7 days at 28 °C. Lipase enzyme production was confirmed after the incidence of UV light under the petri dishes, where the appearance of fluorescent halos around each colony confirmed the hydrolysis of the oil present in the culture medium (Abu-Ruwaida 1991). Experiments were performed in triplicate

Amylase

The amylase enzyme was screened according to the modified method proposed by Andualem (2014). The filamentous fungi isolates were grown in SPDA (Starch Potato Dextrose Agar: 1% soluble starch, 5 g $L^{-1}$of glucose, 15 g L^{-1} of agar in potato infusion). Discs of 0.5 cm in diameter were inoculated in SPDA plates and were incubated for 7 days at 28 °C. After this period, a solution of 1% iodine in 2% potassium iodide was added to the surface of the culture media. The formation of a halo/clear zone around the cells in the culture medium, indicative of starch hydrolysis, was considered as a positive result for extract activity. The experiments were performed in triplicate.

Celullase

Qualitative evaluation of cellulase production was performed according to Teather & Wood (1982), modified. Isolates were grown in culture medium (enrichment) containing: 0.2 g L^{-1} of magnesium sulfate, 0.02 g L^{-1} of calcium chloride, 1 g L^{-1} of monobasic potassium phosphate, 1 g L^{-1} dibasic potassium phosphate, 0.5 g L^{-1} ammonium sulfate, 0.5 g L^{-1} sodium nitrate, 0.5% Carboxymethylcellulose (CMC), 0.6 g L^{-1} yeast extract and 15 g L^{-1} of agar. The filamentous fungi were incubated at 28 °C for 5 days. After 3 days of growth, 5 mL of the 2.5 g L^{-1} Congo Red dye was added to the culture medium containing the samples. After 15 minutes, the plates were washed with 1 M NaCl. Experiments

were performed in triplicate. The formation of a discoloration halo around the cells was considered positive result for celullase activity.

Fungal screening for the ability to decolorize RBBR

Decolourisation halo on solid medium

Screening was performed according to da Silva et al. (2008) modified. All filamentous fungi were grown on PDA media. One fungal culture disc (0.5 cm diameter) from the edge of the colony was transferred to Petri dishes containing the same medium, added of 500 mg L^{-1} and 1000 mg L^{-1} RBBR dye (two different concentrations). Plates were incubated for 21 days at 28 °C. Isolates that showed a discoloration halo around microbial growth were considered as positive results. As control, were used Petri dishes with medium without RBBR, cell free and inoculum without RBBR. All assays were conducted in triplicate.

Decolourisation on liquid medium

The assays to analyze the ability of the microbial strains to discolor RBBR dye in liquid culture medium were performed transferring three fungal mycelium discs (0.5 cm in diameter) from the edge of the colony to erlenmeyer flasks containing PDB culture medium with RBBR added at concentrations 500 mg L^{-1} and 1000 mg L^{-1}. The flasks were incubated in a shaker at 150 rpm for 12 days at 28 °C. Aliquots of 1 mL were collected at 3, 5 and 7 days of incubation, centrifuged at 12.000 rpm for 2 minutes and the supernatant diluted 10 times with distilled water. From these dilutions, it was verified the reduction of absorbance in relation to time zero, in Spectrophotometer at 580 nm wavelength. Erlenmeyers containing medium amended with RBBR, free of cells, and medium with inoculum without RBBR were used as control. All assays were conducted in triplicate. The efficiency of discoloration was expressed by the formula:

$$\text{Decolourisation (\%)} = \frac{A_{\lambda,\ initial} - A_{\lambda,final}}{A_{\lambda,\ initial}} \times 100$$

$A_{\lambda\ initial}$ = initial absorbance and $A_{\lambda\ final}$ = final absorbance

Adsorption assay

RBBR dye adsorption assays were performed using isolates ITF12 (best RBBR bleacher in solid medium) and ITF47 (best RBBR bleacher in liquid medium). The assays were performed as described in the RBBR discoloration test in liquid medium, considering the growth time of 3, 5 and 7 days. The mycelium from each isolate was triturated using Ultraturrax (brand) and spectrophotometer readings were taken before and after grinding. The dye RBBR was used at a concentration of 500 mg mL^{-1}. As control, the culture medium with RBBR without microbial inoculum was used.

Biomass production

Cells recovered in the adsorption assay were used to determine the fungal cell dry mass. A paper filter of 0.22 μm was previously sterilized, dried and weighted. The broth from each fungal was filtered through paper filter by using vacuum pump. Cells were dried at 70 °C for 24 h and the paper filter was weighted again. The weight of the fungal cell dry mass was determined by the weight difference between paper filter before and after filtration.

Enzyme activity assay

Laccase

The quantification of laccase production was performed by spectrophotometric method using

ABTS (22,2 azino-bis ethylbentiazoline). For filamentous fungi, two fungal culture discs (0.5 cm diameter) from the edge of the colony were transferred to 20 mL of 3% Malt Extract medium. Flasks were shaken at 28 °C and at 150 rpm for 7 days. Assays were performed in triplicate. Enzyme extracts were separated from microbial cells by vacuum filtration using 0.22 μm filter paper.

Enzyme activity experiments were performed according to Buswell et al. (1995). A volume of 1.8 μl of each microbial extract was added to 900 μl of acetate buffer and 300 μl of ABTS reagent. The first spectrophotometer reading was performed after the addition of ABTS and a second reading was performed after incubation of the extracts at 28 °C for 10 minutes after the addition of ABTS. ABTS oxidation was measured by monitoring the increase in absorbance at 420 nm. Enzyme activity was defined as the amount of enzyme required to oxidize 1 mol ABTS per min. using an oxidized ABTS value of 3.6x10μ mol.

$$U\ L^{-1} = \frac{(\Delta A)\ (V_t)\ (10^6)}{(t)\ (E)\ (V_s)}$$

Where:
U = enzimatic activity (μmol min^{-1} L^{-1})
ΔA = final absorbance – initial absorbance
V_t = total reaction volume (mL)
10^6 = correction factor (μmol mol^{-1})
t = reaction time (min)
E = molar extinction coefficient (L mol^{-1} cm^{-1})
V_s = sample volume

Cellulase

Quantification of cellulase production was performed with the best positive isolates in the screening test. The method used was performed according to Martinho et al. (2019) by enzymatic activity readings of the isolates extract with the kit Azo-CMCellulase (Megazyme® International, Bray Ireland) modified, in spectrophotometer, following manufacturer specifications. Filamentous fungi isolates were cultivated in 15 mL of MA 3% medium. All cultivation flasks were added of 10 g L^{-1} of cellulose compound (Cefluflok100®). Cell growth was performed for 5 days at 28 °C at 150 rpm. Enzyme evaluation was performed by spectrophotometric method, where 20 μL of each microbial enzyme extract was mixed with 20 μL of 2% Azo-CM-Cellulose.

RESULTS AND DISCUSSION

The isolation of microbial strains from effluent retrieved 45 filamentous fungi. All fungi isolates were submitted to microscopic analysis to identify the taxonomic groups recovered from the sample, and analysis revealed 21 distinct groups, affiliated with species of the genera *Aspergillus* spp. (n = 6), *Penicillium* spp. (n = 5), *Trichoderma* spp. (n = 2), *Cladosporium* sp. (n = 1), *Acremonium* sp. (n= 1) Zygomycetes group (n = 2) and 4 isolates were affiliated with non-morphologically identified taxonomic groups.

Reports in the literature describe the isolation and evaluation of the biotechnological potential of microbial isolates from effluent from the textile industry for their industrial interest. Phatake et al. (2015) carried out a work where it was possible to recover *Aspergillus* spp. strains from textile dye industries waste samples, focusing on textile effluent biodegradation. Praveen & Bhat (2012), performed a study where three fungal species *Penicillium chrysogenum, Aspergillus niger* and *Cladosporium* were isolated from effluent disposal site of the industry located at Peenya, Bangalore (Karnataka) – India. In this study, the authors confirmed the potential of these isolates in decolorize the azo dye Red 3BN and their performance in the treatment of textile effluent. Iordache et al.

(2015) characterized by molecular approaches eight strains isolated from textile post-finishing wastewaters including isolates belonging to the genera *Trichoderma, Polyporus* and *Fusarium*. In the work developed by Salar et al. (2012), three *Aspergillus* spp. strains between them *A, terreus, A. flavus* and *A. niger*, isolated from textile effluent in Panipat, India, were evaluated for their efficient in decolorizing textile azo dyes including Reactive Blue MR, Orange M2R, Yellow M4G, Black HFGR and Red M8B. In this study, the authors reinforced the potential of adapted filamentous fungi from textile effluents for dyes decolorization.

Temperature is of fundamental importance in the development of microbial strains and new genera and species of microorganisms can be identified in underexplored environments with extreme temperature ranges (Margesin & Miteva 2011). The effluent temperature of 28 °C on the day of sampling collection possibly influenced the isolated species, and allowed the recovery of a large number of filamentous fungi affiliated with genera *Aspergillus, Penicillium* and *Trichoderma*. This temperature favored the growth of sporulated filamentous fungi. In addition to temperature, the hydrogen potential (pH) of 7.0 may also have been responsible for selecting species recovered from the textile effluent sample.

Due to the large amount of microorganisms isolated, as well as the diversity of filamentous fungi (21 morphologically distinct strains) recovered from the sample studied, it is possible to state that samples from textile industrial waste are potential sources for the isolation of distinct groups of microorganisms, which may be adapted to the presence of toxic synthetic compounds found in treatment plants.

The overall screenings results for the activities of lipase, cellulose, amylase and ligninolytic enzymes, as well as for RBBR dye discoloration assay, are shown in Table I. From the 45 fungi assayed, 33 (73%) showed activities for the enzymes tested, being able to hydrolyze cellulose (27 isolates), lignin (15 isolates), lipid (8 isolates) and starch (7 isolates). These results were found after the screenings using CMC, guaiacol, olive oil and starch substrates, respectively.

Spectrophotometric analyzes indicated that the cellulolytic enzymatic activities of the six best isolates ranged from 31.01 to 50.69 U mL^{-1}, with isolate ITF 47 being the best producer (Table I). According to Hu et al. (2018), supplementary carbon sources or cellulase inducers may enhance fungal growth and cellulase production. In our work, we used cellulose compound (Cefluflok100®) as inducer of cellulase production.

The search for potentially applicable enzymes in industrial processes using industrial waste as a substrate can be considered an environmentally friendly methodology, in addition to reduce costs of industrial processes due to the usage of substrate as low-cost feedstock (Hu et al. 2018). However, the use of microbial cells recovered from industrial effluents for the production of enzymes of biotechnological interest may be considered an even more promising strategy to explore microbial genetic machinery adapted to the different conditions that the various industrial wastes may present.

Microbial cells isolated from industrial effluents have been studied in search for possible biotechnological applications. According to Khokhar et al. (2012) an isolate of the species *Trichoderma viride*, isolated from a textile effluent, showed hydrolysis activity in culture media supplemented with CMC, demonstrating its cellulolytic potential. Gautam et al. (2011) performed a study where two cellulase producing fungi *Aspergillus niger* and

Table I. **Results of the best enzyme producing strains and/or capable of discoloration of the RBBR dye and their morphological identification.**

Isolates	Activity									Identification
	Lipase	Celullase		Amylase	Laccase		RBBR			
		Halo	Enzymatic activity (UmL^{-1})		Halo	Enzymatic activity (UL^{-1})	Halo decoloration (cm) / concentration (mg L^{-1})	% decoloration / concentration (mg L^{-1})		
ITF 02	+	++								*Penicillium* sp.
ITF 03		+			++			63.6 32.6	500 1000	*Penicillium* sp.
ITF 04		+++	50.14		+++	0.012		63.6 32.6	500 1000	*Penicillium* sp.
ITF 08		+								*Penicillium* sp.
ITF 09		+			+					*Aspergillus* sp.
ITF 10		+++	44.95		+++	0.027		62.2 42.4	500 1000	*Aspergillus* sp.
ITF 11		+								*Penicillium* sp.
ITF 12		+++	50.62				0.8	500		*Penicillium* sp.
ITF 13		+			++					*Trichoderma* sp.
ITF 14		+								NI
ITF 15	+									NI
ITF 18	+									NI
ITF 19	+									NI
ITF 20		+								NI
ITF 21		+		++						*Aspergillus* sp.
ITF 22		+++	3.68	++			0.1	500		*Aspergillus* sp.
ITF 27		+++	36.01	++	++			74.7	500	*Aspergillus sydowii*
ITF 28	+	+								*Penicillium* sp.
ITF 29	+	++								*Trichoderma* sp.
ITF 30	+	+++	46.74	+	+++	0.026	0.1	500		*Aspergillus sydowii*
ITF 31		+						63.3	500	NI
ITF 33		++						64.9 44.9	500 1000	*Acremonium* sp.
ITF 34		+		++						*Penicillium* sp.
ITF 35				++						*Penicillium* sp.
ITF 36				+						NI
ITF 39		++								*Penicillium* sp.
ITF 40		+								*Penicillium* sp.
ITF 42	+									NI
ITF 43		+			+					Zygomicete
ITF 44		+								*Penicillium* sp.
ITF 45		++								NI
ITF 47		+++	50.69		++			85.3 33.5	500 1000	*Aspergillus* sp.
ITF 60		+								*Penicillium* sp.

+ = discrete activity in solid culture medium.
++ = moderate activity in solid culture medium.
+++ = good activity in solid culture medium.
ITF = textile industry fungi.
ITF 27 and 30 = isolates were identified by molecular approaches (Bernal et al. 2021).

Trichoderma sp., isolated from municipal solid waste were able to produce about 2.1 U mL^{-1} of cellulase, when the authors adjusted the best pH and temperature conditions (6-7 and 40°C, respectively). Imran et al. (2018) characterized the production of a commercial cellulose produced by *Aspergillus niger* IMMIS1. In this work, the authors isolate twenty three species of fungi between then *Aspergillus, Trichoderma* and *Penicillium*, collected from different samples including textile effluents and wastes agriculture. The *A. niger* IMMIS1 strain was able to produce 400 and 500 U mL^{-1} when the authors adjusted the best pH and temperature conditions (4.5 and 35°C, respectively).

The guaiacol induction assay for identification of ligninolytic activity revealed that 21.4% of the filamentous fungi were able to produce a brownish halo in solid medium amended with guaiacol. The characteristic change of culture medium to a dark shade, with evident brown halos of phenol oxidase activity was considered positive in 9 fungal isolates (Table I). Concerning laccase production, the best producer was the ITF10 fungus, which produced 0.0275 U L^{-1} of enzyme (Table I). From these results, it is possible to observe that screening assays for ligninolytic enzymes production using guaiacol can produce false positive hits. Nine potential laccase-producing filamentous fungi in medium supplemented with guaiacol were found, however, the enzymatic activity assays using the compound ABTS, a substrate with higher selectivity of laccase enzyme, revealed only 3 enzyme producing isolates. Atalla et al. 2013, demonstrated that ABTS has higher laccase specificity than guaiacol, using the laccase enzyme produced by *Trematosphaeria mangrovei* and the relative activity to ABTS on 5 mM was 100% against 10.66% of guaicol in the same concentration.

Although fungi from the Basidiomycota phylum may synthesize a large amount of laccase, isolates belonging to Ascomycota phylum have already been evaluated for laccase production. According to Passarini et al. (2015), *Nigrospora* sp. (CBMAI 1328) and *Arthopyrenia* sp. (CBMAI 1330) strains, isolated from marine sponge were able to produce 1.2 and 3.9 U L^{-1} of enzyme after 7 days, respectively. Singh et al. (2010) demonstrated the laccase production of 0.0029 U L^{-1} by *Aspergillus heteromorphus* isolated from distillery spent wash, when the strain was grown up on sugarcane baggase. These results demonstrate the potential laccase producer from ascomycete fungi from different environmental sources.

Industrial effluents contain a wide variety of compounds ranging from synthetic dyes used in dyeing processes as well as high concentrations of different inorganic compounds including salts (Upadhyay et al. 2016) and hydrocarbons (Ghaly et al. 2014) which makes the use of halotolerant microorganisms for industrial wastewater bioremediation processes an advantageous strategy (Divya et al. 2014). Filamentous fungi recovered from textile industry wastewater able to produce hydrolytic enzymes such a lipase, amylase, cellulase and laccase have become a very applicable bioremediation tool (Divya et al. 2014, Dhiman & Shirkot 2015).

Lipase production assay retrieved 8 filamentous fungi (19%) able to produce fluorescent halos in media added of Rhodamine B (Table I). Industrial wastes constitute a significant source for isolation of new organisms (Ertugrul et al. 2007). The need to explore industrial effluents in an attempt to find sustainable alternatives to the biotechnology industry is necessary. Greice et al. (2016) performed a work with microorganisms isolated from different food industries effluents in the region of Pelotas, south Brazil, and their lipase

production was evaluated by using Rhodamine B as substrate. Twenty-one bacteria and seven filamentous fungi were isolated and 71.43% of the bacteria and 57.14% of the fungi were able to produce extracellular lipase. The best lipase producer found was *E. aerogenes* with enzymatic activity of 1.54 UmL^{-1}.

Works has been demonstrated that the RBBR dye, a compound derived from anthracene (polycyclic aromatic hydrocarbon), can be considered an efficient screening method for microorganism including filamentous fungi that are able to degrade recalcitrant pollutants (Vitali et al. 2006, Passarini et al. 2011). Ligninolytic fungi may producing a non-specific enzymatic extracellular complex that include the laccase, lignin peroxidase and manganese peroxidase enzymes able to oxidize polycyclic aromatic hydrocarbon and others related compounds including aromatic dyes (Bonugli-Santos et al. 2010).

Thus, regarding the RBBR dye discoloration assays, only 3 fungi (7%) showed discoloration in the solid medium assays. The ITF 12 was the best isolate to decolorize the dye with a 0.8 cm halo formation at a concentration of 500 mg L^{-1}. All filamentous fungi were submitted to quantitative tests for discoloration of RBBR in liquid medium, and 18 (42.8%) and 13 (31%) isolates showed satisfactory results of RBBR discoloration at 500 mg L^{-1} and 1000 mg L^{-1} concentrations, respectively. The ITF 47 was the best isolate to decolorize the dye in liquid medium, in all time and concentration studied (Figure 1).

Overall, after seven days of growth, there was a significant discoloration of the RBBR dye at both concentrations tested, i.e. 500 and 1000 mg L^{-1} (Figure 1). Seven filamentous fungi showed discoloration capacity of above 60%, with a discoloration range between 62.5% to 85.3% in the concentration of 500 mg L^{-1} (ITF 03, 04, 10, 27, 21, 33 and 47) while at dye concentration of 1000 mg L^{-1}, five filamentous fungi showed discoloration ability percentages above 30%, with a variation of 32.6 to 44.9% (ITF 03, 04, 10, 33 and 47). Four isolates (ITF 03, 04, 33 and 47) showed the ability to discolor the RBBR dye at both concentrations tested and with discoloration rates up to 60% and 30% for 500 mg L^{-1} and 1000 mg L^{-1} concentrations, respectively.

Studies have demonstrated the potential that microorganisms isolates from textile industrial samples may present as sources

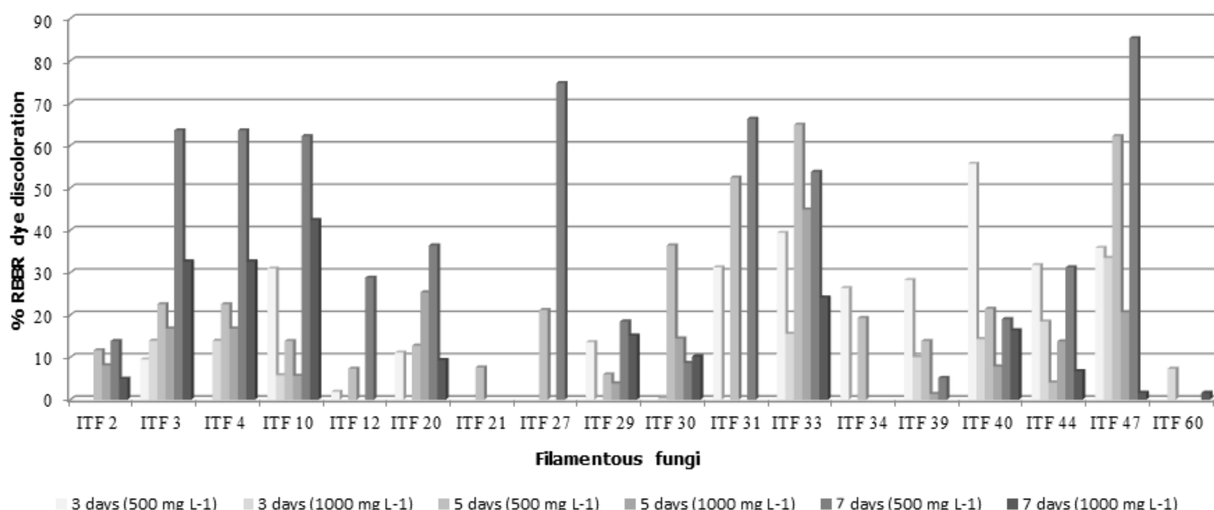

Figure 1. Percentage of discoloration (500 mg L^{-1} and 1000 mg L^{-1}) of RBBR dye by fungal isolates.

of biotechnologically active compounds. In the work developed by Pereira et al. (2010), filamentous fungi from an industrial textile effluent treatment plant were isolated and assayed to evaluate the discoloration of synthetic dyes RBBR, Yellow Reactive 145, Red Reactive 195, and the textile waste recovered from the company. Among all isolates, authors found that isolate *Geothricum candidum* was able to discolor the industrial effluent.

The agitation of the culture medium in the shaker favored the microbial growth and consequently improved the performance of the isolates in the discoloration process of the dye. Similar results were found by Rani et al. (2014), by using the strains *Phanerochaete chrysosporium* and *Aspergillus niger* isolated from dyes contaminated soil, in discoloration experiments using Basic Fuchsin, Nigrosin, Malachite Green and a dye mixture. *A. niger* recorded maximum decolorization of the Basic Fuchsin dye (81.85%) and, on the other hand, *P. chrysosporium* showed maximum Nigrosin decolorization (90.15%). The selected fungal strains had their performance enhanced under shaking conditions compared to stationary method.

To verify if the discoloration of the RBBR occurred due to the enzymatic action or due to the adsorption of the dye by the mycelia, a RBBR dye adsorption test was performed with fungal mycelium, using the two isolates that showed better RBBR discoloration results, ITF 12 (halo of 0.8 cm on 500 mg L^{-1} of RBBR) and ITF 47 (RBBR discoloration of 85.3% and 33.5% on 500 mg L^{-1} and 500 mg L^{-1} of RBBR, respectively).

Both isolates showed satisfactory cell growth, ranging from 0.025 to 0.066 mg mL^{-1} and 0.02 to 0.02 to 0.069 mg mL^{-1} for samples ITF 12 and ITF 47, respectively, and biosorption of RBBR dye by fungal mycelia was observed. The ITF 47 isolate, which showed the best dye discoloration at the concentration of 500 mg mL^{-1} revealed absorption of 26.27% of the dye by its mycelia (the initial discoloration after 7 days of growth in the adsorption test was 38.94%). However, fungal mycelium adsorption may have advantages in the application of microbial cells in bioremediation processes of environmental pollutants, including synthetic dyes.

Results obtained in the present work demonstrated the biotechnological potential that filamentous fungi recovered from textile wastewater treatment plant samples may present. Fungi inhabiting this environment may produce several enzymes of industrial interest including lipases, cellulases, amylases and laccases, for use in synthetic dye bleaching processes. Further research is needed to reveal the importance of microbial communities present in industrial effluent samples and their dynamics within such harsh environment. The characterization of enzymes found, using different microbial growth conditions as well as to optimize, purify and apply them in pilot scale industrial processes will be the next stage of the work, as well as *in situ* bioremediation processes.

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SPFB, MMAL, JJ-B, PEG, and EB carried out the analyses of the data; JRO and MRZP wrote, reviewed and edited the manuscript; all authors approved the final version of this manuscript.