Brazilian Journal of Microbiology (2006) 37:481-487
ISSN 1517-8283

BIODEGRADATION OF REACTIVE TEXTILE DYES BY BASIDIOMYCETOUS FUNGI FROM BRAZILIAN ECOSYSTEMS

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Submitted: November 25, 2005; Returned to authors for corrections: March 13, 2006; Approved: July 18, 2006

ABSTRACT

The potential of *Trametes villosa* and *Pycnoporus sanguineus* to decolorize reactive textile dyes used for cotton manufacturing in the State of Minas Gerais, Brazil, was evaluated. Growth and decolorization halos were determined on malt extract agar containing 0.002g L^{-1} of the dye. *T. villosa* decolorized all 28 of the tested dyes while *P. sanguineus* decolorized only 9. The effect of culture conditions (shaking and dye and nitrogen concentration) on the degradation of Drimaren Brilliant Blue dye was evaluated during growth of the fungi in liquid synthetic medium. Shaking favored degradation and decolorization was not repressed by nitrogen. In pure culture, *T. villosa* and *P. sanguineus* decolorized synthetic effluent consisting of a mixture of 10 dyes. Higher decolorization of the synthetic effluent was observed when a mixed culture of the two fungi was used. This study demonstrated differences between tropical basidiomycete species in terms of their ability to degrade reactive dyes, and reinforces the potential of this group of fungi for the decolorization of textile effluents.

Key words: textile industry, synthetic effluent, reactive dyes

INTRODUCTION

In the State of Minas Gerais, the second largest textile pole in Brazil, most textile industries operate with pure cotton fibers or cotton fibers mixed with polyester. Few of these factories possess their own wastewater treatment plant, and most of the time the effluents are discharged into the water bodies without any treatment. In general, the difficulties encountered in the wastewater treatment resulting from dyeing operations lies in the wide variability of the dyes used and in the excessive color of the effluents. Many dyes and other substances present in textile effluents are not readily degraded during their permanency in traditional aerobic treatment systems (17,26). Although many physicochemical techniques of decolorization have been developed over the last 20 years, few have been implemented by the textile industries due to their high cost, low efficiency and inapplicability to a wide variety of dyes. A definitive solution of the color problem of textile effluents would provide a marked competitive advantage for this industrial sector. Since no single process is able to decolorize all textile effluents, a solution for each situation should be considered, possibly involving a combination of different methods (1).

The success of a biological process for color removal from a given effluent depends in part on the utilization of microorganisms that effectively decolorize synthetic dyes of different chemical structures. Many bacteria, actinomycetes, yeast and mitosporic fungi are able to decolorize dyes, with color removal by these microorganisms being mainly attributed to adsorption of the dyes (1,27).

Basidiomycetous fungi are able not only to decolorize but also to degrade and mineralize a broad spectrum of different dye structures (azo, anthraquinone, heterocyclic, triphenylmethane

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and polymeric dyes), in addition to numerous other toxic organic and recalcitrant compounds. The enzymatic system involved in the degradation of pollutants by these fungi is nonspecific and even acts on mixtures of pollutants (12,23,26).

The objectives of the present study were 1) to evaluate the potential of native Brazilian basidiomycetes to degrade a broad spectrum of reactive synthetic dyes, 2) to analyze the decolorization of reactive dyes under different culture conditions (shaking and dye and nitrogen concentration), and 3) to determine the decolorization of a synthetic effluent by pure and mixed cultures of basidiomycetes.

MATERIALS AND METHODS

Fungi

Trametes villosa (Fr.) Kreisel CCB176, with known ligninolytic activity (16), was obtained from the Basidiomycetes Culture Collection (CCB) of the Institute of Botany, São Paulo. *Pycnoporus sanguineus* (L.: Fr.) Murr. UFMGCB03 was isolated from the biological reserve of the Museum of National History, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais (20). The cultures were maintained on 2% (w/v) malt extract agar (MEA) at 4ºC.

Dyes

The reactive dyes (Table 1) were provided by Cia. Manufatora de Tecidos de Algodão (Cataguases, MG) and are routinely used in the dyeing process of cotton fibers.

Dye decolorization on solid medium

A disc (∅ 2 mm) of fungal mycelium in MEA was inoculated into the center of Petri dishes (∅ 90 mm) containing MEA and 0.002 g L^{-1} of the dye, in triplicate. The plates were incubated at 28ºC in the dark until they were completely colonized with the fungus or for a maximum period of 21 days. The diameters (cm) of the decolorization and growth halos were determined in two perpendicular directions of the plate (13). Plates containing the dye but not inoculated served as control.

Decolorization of Drimaren Brilliant Blue dye in liquid medium

Three discs (∅ 2 mm) of *T. villosa* on MEA were transferred to 250 mL Erlenmeyer flasks containing 50 mL 2% malt extract broth (MEC) supplemented with 0.002 to 0.01 g L^{-1} of Drimaren Brilliant Blue, in duplicate. The flasks were incubated in the dark at 28ºC for 21 days. Non-inoculated culture medium was used as control. The effect of shaking (130 rpm) on decolorization was evaluated at a dye concentration of 0.002 g L^{-1}.

Influence of nitrogen on the decolorization of Drimaren Brilliant Blue

Three discs (∅ 2 mm) containing the fungus grown on 2% MEA at 28ºC were transferred to 250 mL flasks containing 50 mL modified basal medium (10) supplemented with 0.002 g L^{-1} Drimaren Brilliant Blue. The composition of the medium (per liter) was: 10 g glucose, 0.001 g thiamine-HCl, 1 g KH_2PO_4, 0.5 g $MgSO_4$, 0.01 g $CaCl_2$, 0.01 g $FeSO_4 7H_2O$, 0.001 g $MnSO_4 4H_2O$, 10 mL sodium acetate buffer, pH 4.5, and ammonium tartrate at the concentration needed to obtain 1.2, 12 and 22.4 mM nitrogen. The flasks were incubated at 28ºC and 130 rpm. The experiments were carried out in duplicate. Non-inoculated culture medium was used as control.

Decolorization of synthetic effluent

The synthetic effluent consisted of a mixture of 10 dyes (0.001g L^{-1}): Remazol Brilliant Orange 1, Levafix Gold Yellow 10, Procion Yellow 14, Drimaren Brilliant Blue 17, Remazol Brilliant Blue 18, Cibacron Black 55, Procion Black 59, Drimaren Turquoise Blue 62, Drimaren Brilliant Red 67, and Remazol Red 75. Two discs of fungal mycelium in MEA were inoculated into flasks containing 100 mL MEC supplemented with the synthetic effluent at a final concentration of 0.01 g L^{-1}. For mixed cultures, one growth disc containing mycelium of each fungus was used. The flasks were incubated at 28ºC and 130 rpm. The fungi were also grown in culture medium without the synthetic effluent.

Degradation of Drimaren Brilliant Blue

The content of the flasks was removed at determined time intervals and filtered (0.45 µ) and the filtrate diluted 1/10 was used to determine dye degradation (4). Decolorization was analyzed by determining the absorbance at 590 nm and is expressed as relative percentage taking the non-inoculated control as 100%. The absorbance spectrum of the dye was determined in the visible range (400 to 700 nm) with a Hitachi U-3000 spectrophotometer.

Glucose and biomass

Glucose concentration was assayed by the method of Luff-Schoorl (19). Biomass was determined as dry weight after drying the samples at 60ºC for 48 hours.

RESULTS AND DISCUSSION

T. villosa and *P. sanguineus* were analyzed regarding their ability to decolorize 28 reactive dyes routinely employed in cotton dyeing (Table 1). *T. villosa* decolorized all dyes tested, whereas 5 dyes were not decolorized by *P. sanguineus*. *T. villosa* completely decolorized 17 dyes, whereas *P. sanguineus* completely decolorized only 8. Some dyes inhibited fungal growth, with a reduction in the growth rate. In general, dye decolorization progressed more slowly than radial growth and the decolorization halo of some dyes did not occupy the entire diameter of the plate even when the incubation time was prolonged to 21 days. Levafix Orange, Drimaren Brilliant Orange, Drimaren Red and Levafix Red were found to be more resistant

Table 1. Decolorization of reactive dyes by *Trametes villosa* CCB176 and *Pycnoporus sanguineus* UFMGCB03 on malt extract agar containing 0.002 g L^{-1} of the dye.

Dyes[1]	Chemical class of dye[2]	*Pycnoporus sanguineu*		*Trametes villosa*	
		Time (days)[3]	Decolorization[4]	Time (days)	Decolorization
Cibacron Black 55	MCT+VS	10	D (21)	nd	D (10)
Cibacron Blue 19	MFT	10	P (21)	10	P (14)
Cibacron Blue 20	MCT+VS	10	D (14)	10	D (10)
Cibacron Deep Blue 50	VS	7	D (14)	7	D (10)
Cibacron Orange 46	MCT+VS	10	P (14)	10	P (10)
Drimaren Black 58	VS	7	D (21)	7	D (14)
Drimaren Blue 21	VS	7	D (10)	7	P (17)
Drimaren Brilliant Blue 17	VS	7	D (7)	nd	P (12)
Drimaren Brilliant Red 67	MCT	7	no	nd	D (14)
Drimaren Deep Blue 25	FCP	7	D (10)	7	D (10)
Drimaren Deep Blue 26	MCT	21	P (HI)	10	D (10)
Drimaren Red 40	MCT	7	P (HI)	7	D (17)
Drimaren Red 70	MCT	10	no	7	P (HI)
Drimaren Remazol Orange 45	MCT	10	P (HI)	7	P (HI)
Drimaren Turquoise Blue 62	MCT	7	P (10)	nd	D (10)
Levafix Brown 37	DCQ	7	no	7	P (17)
Levafix Gol Yellow 10	FCP	10	P (21)	nd	D (10)
Levafix Orange 2	FCP	10	P (HI)	7	P (HI)
Levafix Red 42	FCP	7	P (HI)	7	D (14)
Levafix Red 73	FCP	10	no	10	P (HI)
Levafix Royal Blue 31	VS	10	P (10)	7	D (14)
Procion Black 59	MCT	10	P (HI)	nd	D (14)
Procion Blue 29	DCT	7	P (10)	10	P (10)
Procion Blue 30	MCT	10	P (10)	7	P (10)
Procion Yellow 14	MCT	10	no	nd	D (14)
Remazol Brilliant Blue 18	VS	7	D (10)	5	D (5)
Remazol Brilliant Orange 1	VS	7	P (HI)	nd	D (7)
Remazol Red 75	MCT+VS	7	P (HI)	nd	D (10)
Control [5]	-	7	-	7	-

1. Names provided by Cia. Manufatora de Tecidos de Algodão; 2. Chemical class: VS = vinyl sulfone; DCP = dichloropyrimidine; DCQ = dichloroquinoxaline; FCP = fluorochloropyrimidine; MCT = monochlorotriazine; DCT = dichlorotriazine; MFT = monofluorotriazine; TCP = trichloropyrimidine; 3. Time (in days) necessary for the fungus to occupy the whole Petri dish; 4. D: complete decolorization; P: partial decolorization; no: no decolorization; nd: not determined; HI: decolorization halo that did not occupy the entire diameter of the Petri dish. The time in days spent to decolorize the whole Petri dish is given in parentheses; 5. Control: growth of the fungus in the absence of dye.

to fungal action, probably because of their chemical structure and the presence of chlorine in the molecule (22,24).

Dye decolorization by fungi during growth on solid medium has been widely employed to identify the ligninolytic potential and potential degradation of xenobiotic compounds by basidiomycetes (13,23,26). The time required by *T. villosa* and *P. sanguineus* to decolorize the synthetic dyes was similar to that reported for other basidiomycetes (7 to 20 days). Differences in the capacity of dye decolorization between fungi have been related to inter- and intraspecific variations, the molecular complexity of the dye and culture conditions (6,15,25).

The action of *T. villosa* on a broad spectrum of textile dyes confirms the previously demonstrated biotechnological potential of this fungus (12,16). *P. sanguineus* decolorized Drimaren Brilliant Blue, which *T. villosa* was unable to decolorize completely on solid medium. Therefore, some parameters that

influence the decolorization of dyes by basidiomycetes were studied in submerged culture using this dye and pure and mixed cultures of the two fungi.

Shaking favored the decolorization of Drimaren Brilliant Blue by *T. villosa* (Fig. 1), with about 85% decolorization being obtained after seven days. Although shaking has been shown to suppress the expression of the ligninolytic system in *Phanerochaete chrysosporium* (10,26), this condition generally results in higher dye decolorization than obtained with static cultures due to an increase in mass and oxygen transfer between cells and the medium, factors that optimize the action of oxidative enzymes. Thus, shaking (200 rpm) has been found to significantly increase dye decolorization by *P. chrysosporium*, *Trametes versicolor* and *Bjerkandera* sp. BOS55 (25). Shaking cultures (180 rpm) of *Bjerkandera fumosa*, *Kuehneromyces mutabilis* and *Strofaria rugoso-annulata* promoted a higher dye decolorization than static cultures (7). However, Kirby et al. (9) observed better dye decolorization by *Phlebia tremellosa* under static conditions, but decolorization was only determined after 14 days of incubation and therefore monitoring of the decolorization kinetics was not possible.

The initial concentration of the dye influenced the decolorization capacity of *T. villosa* (Fig. 2). At seven days, decolorization rates of 34 and 55% were observed at dye concentrations of 0.002 and 0.004 g L^{-1}, respectively, and about 70% decolorization was obtained at concentrations higher than 0.006 g L^{-1}. After 21 days of culture, no significant difference in decolorization rates was observed between the various dye concentrations tested. Higher decolorization rates of Remazol Brilliant Blue R were obtained with increasing concentrations of the dye (2,18).

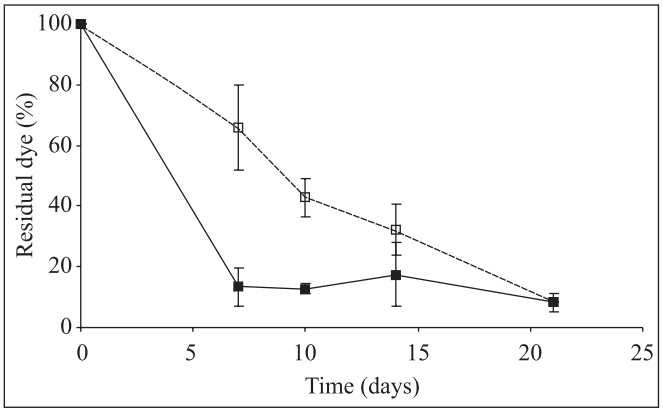

Figure 1. Decolorization of Drimaren Brilliant Blue dye (0.002 g l^{-1}) by *Trametes villosa* CCB176 grown in the static condition (□) and under shaking at 130 rpm (■). Decolorization was calculated considering the color of the non-inoculated control to be 100%. The bars indicate the standard deviation between duplicate experiments.

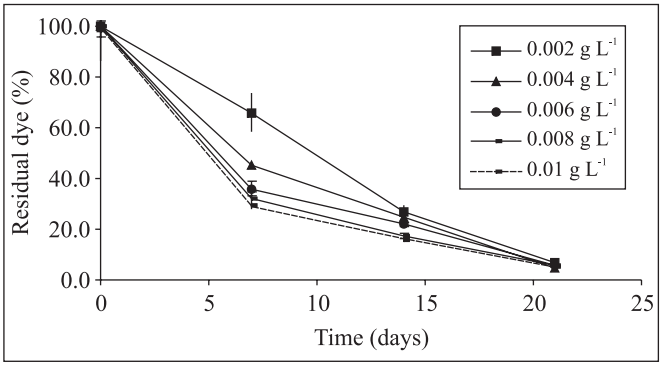

Figure 2. Decolorization of Drimaren Brilliant Blue dye by *Trametes villosa* CCB176 grown in the presence of different initial concentrations of the dye. Decolorization was calculated considering the color of the non-inoculated control to be 100%. The bars indicate the standard deviation between duplicate experiments.

The nitrogen concentration in the culture medium influenced the growth of *T. villosa* and *P. sanguineus*, with a greater biomass being obtained in the condition of nitrogen sufficiency (Fig. 3). However, decolorization of Drimaren Brilliant Blue by these fungi did not depend on the initial nitrogen concentration, with 100% decolorization being obtained after 7 days of incubation irrespective of the initial nitrogen concentration. Degradation of the dye was confirmed based on its absorption spectrum. Therefore, in contrast to *P. chrysosporium*, *Trametes versicolor*, *Phlebia brevispora* and *Lentinula edodes* (3,5,11), *T. villosa* and *P. sanguineus* belong to a group of fungi whose ligninolytic systems are not regulated by nitrogen concentration, as also observed for *Pleurotus ostreatus* and *Ceriporiopsis subvermispora* (11,14,21).

The presence of synthetic effluent did not influence the growth of *T. villosa*, but stimulated glucose consumption by this fungus after seven days of culture (Fig. 4). Approximately 90% decolorization was obtained after 21 days, as also confirmed by the absorption spectrum. The higher glucose consumption by the fungus in the presence of synthetic effluent might be evidence of increased metabolism due to the detoxification mechanism. The presence of the effluent stimulated the growth of *P. sanguineus*, with an approximately 0.5 g higher biomass compared to control. Since glucose consumption by this fungus was not influenced by the presence of the effluent, the higher biomass observed might indicate the utilization of the dyes as a carbon source for growth. During the first days of culture, *P. sanguineus* decolorized the effluent more rapidly than *T. villosa*, with approximately 80% decolorization being observed after 10 days (Fig. 4).

In general, significant decolorization rates of synthetic mixtures of reactive dyes have been reported for basidiomycetes. *P. chrysosporium* completely decolorized a synthetic effluent

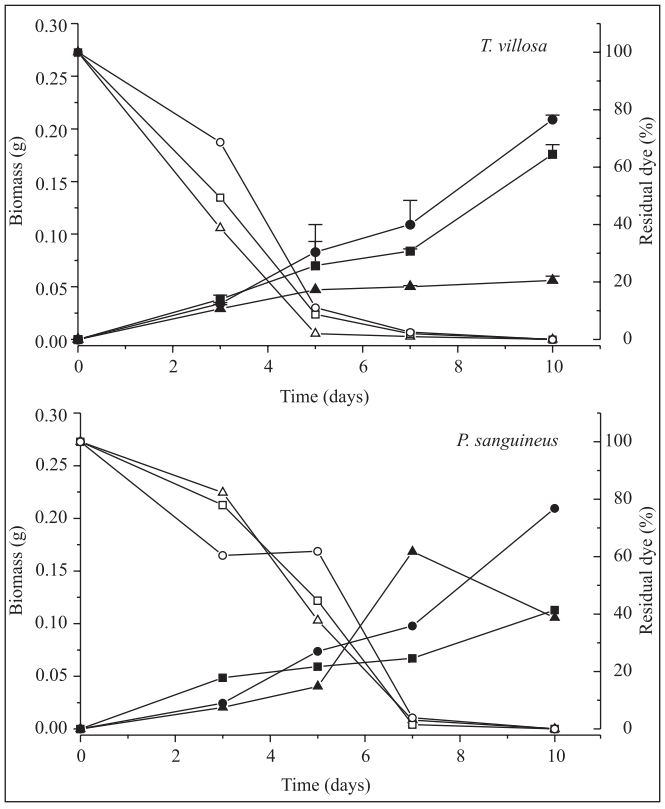

Figure 3. Decolorization of Drimaren Brilliant Blue dye by *Trametes villosa* CCB176 and *Pycnoporus sanguineus* UFMGCB03 grown in the presence of different nitrogen concentrations: (△, ▲) 1.2 mM; (□, ■) 12 mM; (○, ●) 22.4 mM. Biomass (filled symbols), decolorization (open symbols). Decolorization was calculated considering the color of the non-inoculated control to be 100%. The bars indicate the standard deviation between duplicate experiments.

consisting of nine dyes (final concentration of 0.5 g L^{-1}) within seven days of culture (8). *Phlebia tremellosa* reduced by 96% the color of a synthetic effluent consisting of a mixture of eight reactive dyes (final concentration of 0.2 mg L^{-1}) after 14 days of stationary culture (9).

In the mixed culture of *P. sanguineus* and *T. villosa*, a greater biomass was obtained in the presence of the synthetic effluent and no glucose exhaustion was observed. Dye decolorization was faster in the mixed culture compared to the pure culture, with approximately 80% and 90% color reduction at 7 and 17 days, respectively (Fig. 5). These results support the importance of studies involving mixed cultures in order to better understand the possible relationship between different ligninolytic systems in the degradation of pollutant molecules.

The present results demonstrated differences between Brazilian basidiomycetous fungi grown in pure or mixed

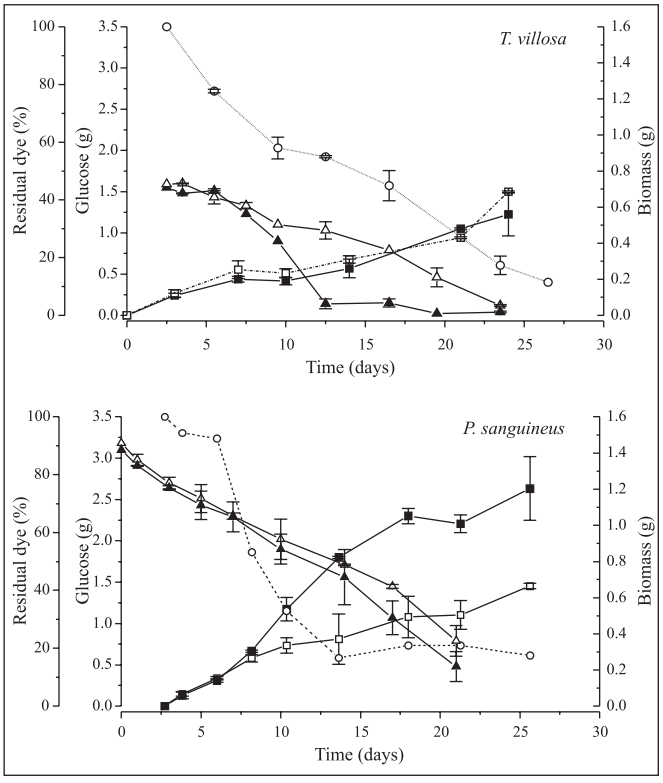

Figure 4. Growth of *Trametes villosa* CCB76 and *Pycnoporus sanguineus* UFMGCB03 in the presence of synthetic effluent. (■) Biomass, (○) residual color, (▲) glucose consumption. Control: growth in the absence of dye. (□) Biomass and (△) glucose consumption. The bars indicate the standard deviation between duplicate experiments.

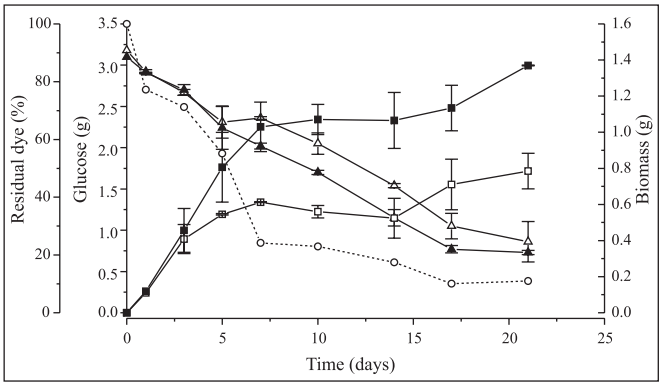

Figure 5. Growth of a mixed culture of *Trametes villosa* CCB76 and *Pycnoporus sanguineus* UFMGCB03 in the presence of synthetic effluent. (■) Biomass, (○) residual color, (▲) glucose consumption. Control: growth in the absence of dye. (□) Biomass and (△) glucose consumption. The bars indicate the standard deviation between duplicate experiments.

cultures in terms of their ability to degrade reactive textile dyes. The excellent performance of *T. villosa* and *P. sanguineus* in the decolorization of dyes of different chemical structures reinforces the potential of these fungi for environmental decontamination.

ACKNOWLEDGMENTS

This study was supported by CETEC. We are indebted to Cia de Tecidos Cataguases for providing the dyes and to Dr. Dácio R. Matheus for revising the manuscript.

RESUMO

Biodegradação de corantes têxteis reativos fungos basidiomicetos do ecossistema brasileiro

O potencial de *Trametes villosa* e *Pycnoporus sanguineus* de descolorir corantes têxteis reativos utilizados na manufatura de algodão no estado de Minas Gerais foi avaliado. Halos de crescimento e descoloração foram determinados em agar extrato malte (MEA) com 0,002g L^{-1} do corante. *T. villosa* descoloriu os 28 corantes testados e *P. sanguineus* apenas 9. A influência de condições de cultivo (agitação, concentração de corante e concentração de nitrogênio) na degradação do corante azul brilhante Drimaren foi avaliada durante crescimento dos fungos em meio líquido sintético. Agitação favoreceu a degradação e não foi observada repressão da descoloração pelo nitrogênio. Em cultura pura, *T. villosa* e *P. sanguineus* descoloriram efluente sintético constituído por uma mistura de dez corantes. Maior descoloração do efluente sintético foi observada no cultivo misto destes dois fungos. Este estudo evidenciou diferenças entre espécies de basidiomicetos tropicais na capacidade em degradar corantes reativos e reforçou o potencial deste grupo de fungos para a descoloração de efluentes têxteis.

Palavras-chave: indústria têxtil, efluente sintético, corantes reativos

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