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Full Length Research Paper

Decolorization of azo dyes by *Pycnoporus sanguineus* and *Trametes membranacea*

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In the present work, decolorization of dyes Orange II and Black V by the fungi *Pycnoporus sanguineus* and *Trametes membranacea* was assessed at six, 12 and 18 days, through fractional design, with a total of 16 trials, statistically represented by 2^{6-2}. The fungi were grown in Erlenmeyer flasks containing the malt and King media supplemented with 0.05% m/v of the dyes Orange II and Black V, respectively under pH 4.5 and 5.0 in the presence or absence of agitation and/or luminosity. The fungal species showed different behaviors on the biomass production and decolorization of the dyes under different growing conditions. *P. sanguineus* showed the highest production of biomass (7.5 g/l) when grown on King medium supplemented with dye Black V, under the absence or presence of agitation, luminosity and at pH 4.5 and 5.0, while *T. membranacea* showed a 7.5 g/l of biomass in all growing conditions for the two dyes tested. As for the agitation of the flasks, the rotation speed of 130 rpm was the best condition for color removal. The fungi studied reached a decolorization percentage of over 50% for the dyes in all flasks under agitation.

Key words: Basidiomycetes, white rot fungi, azo dyes, decolorizations.

INTRODUCTION

Most of the produced dyes go to the textile industry, although the leather or paper, food, cosmetics, paints and plastics industries are also important users (Minatti, 2005; Aksu, 2005). Half of the dyes used today are of the azo kind (Zanoni and Carneiro, 2001). Azo dyes have in common, the group-N = N- called "azo". The reaction of nitrous acid (HONO) with an Ar-NH_2 aniline results in the diazonium ion Ar-N = N^+, which reacts quickly with other anilines or phenols to form azo compounds.According to the Brazilian Association of Chemical Industry- ABIQUIM (2006), dyes and pigments can be classified according to the chemical classes they belong and the applications they are destined to have. For example, the azo chemical class may be divided according to their application in acid, direct, disperse, basic, mordant and reactive dyes. The reactive azo dyes have been the cause of major environmental concern due to its intense use in the textile industry (Balan, 2002) and are considered recalcitrant and highly toxic, and may have carcinogenic and genotoxic properties when present in any living organism (Al-Sabti, 2000; Guaratini and Zanoni, 2000; Gnanamani et al., 2004). Although, decolorization is a much challenging process for both the textile industry and systems of wastewater treatment, there is a great potential for the systems development of microbiological decolorization with the total mineralization of dyes. Within this process, the serious wood destroying Basidiomycetes has been shown to have strong oxidative activity and low specificity of their ligninolytic enzymes to the substrate (Kamida and Durrant, 2005; Seys and Aksoz, 2005; Da Paz et al., 2010).

The great biotechnological potential of white rot fungi is directly related to its capability to degrade a wide variety

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Table 1. Chemical features of dyes investigated in this work.

Dye	Ionization	λ(nm) [a]	Chemical class	Empirical formula	Molecular weight (g/mol)
Orange II	Acid	485	Azo	$C_{16}H_{11}N_2NaO_4S$	350.32
Black V	Acid	595	Diazo	$C_{26}H_{21}N_5Na_4O_{19}S_6$	991.82

[a]Maximum absorption.

Table 2. Factor levels used for the 26-2 fractional factorial designs.

Level	Factor	
Fungi	*T. membranacea*	*P. sanguineus*
Culture media	Malt	King
Shaker (rev/min)	0	130
Lighting regime	dark	light
pH	4.5	5.5
Dye	Orange II	Black V

of textile wastewater, especially synthetic dyes. The ability of these fungi to decolorize various types of dyes due the action of their extracellular enzymes such as laccase, lignin peroxidase and manganese peroxidase was reported (Tuor et al., 1995; Sevil and Buket, 2011). According to Lyra et al. (2009), the decolorization of dyes could be used as a simple and effective way to indirectly select fungi producers of lignocellulolytic enzymes. However, fungi are affected by environmental conditions. Since there are many factors that influence the development of fungi, it is extremely importance to know the influence and effects of growing conditions of these organisms, to obtain greater efficiency in the process of decolorization of industrial pollutants.

This study aimed to evaluate the potential of *Pycnoporus sanguineus* and *Trametes membranacea* in the decolorization of azo dyes (Orange II and Black V) under different growing conditions (pH, shaking speed, light and culture media).

MATERIALS AND METHODS

The species *T. membranacea* and *P. sanguineus* were collected and isolated in the Atlantic Rain Forest in Pernambuco, Brazil. Collection, preparation of the material and micro- and macro-scopic analysis were done following the usual methods for these fungi (Singer, 1951; Teixeira, 1955). For identification, the following literatures were used: Cavalcanti (1976) and Gugliotta (1997). The fungal cultures were isolated and grown at 28°C in glass tubes containing culture media agar-malt.

The following synthetic dyes were used: Orange II (Sigma, St. Louis, MO, USA) and Black V (Suape Têxtil S/A, Pernambuco, Brazil) (Table 1). Their main chemical characteristics are summarized in Table 1.

Cultural conditions

For the aliquots of 2 cm^2 of mycelium, each of the aliquots that was isolated was seeded in agar-malt medium and grown for 10 days in 125 ml Erlenmeyer flasks containing 30 ml of King medium (Silva et al., 2003) and malt medium (20 g/l), in pH 4.5 and 5.5, after which 0.05% w/v of each selected dye was added. Then, they were incubated in orbital shaker (130 and 0 rev/min) at 28°C, for six, 12 and 18 days (Table 2). After this period of time, the mycelium was separated by filtration through membranes with 0.45 µm-pore diameter (Millipore, Barueri, SP, Brazil). The filtrate was used to evaluate the degree of decolorization of each dye. Each treatment was carried out twice, using as control, the same medium without any inoculation that was presented for the same operations. The determination of biomass was estimated gravimetrically using the oven at 70°C until constant weight.

Color reduction measurements

The color reduction was determined by means of a spectro-photometer, ultraviolet–visible spectroscopy (UV–VIS), model B582 (Micronal, São Paulo, SP, Brazil) by optical density measurements at the wavelengths listed in Table 1. The decolorization efficiency was calculated according to the equation: decolorization (%) = (A - B/A) × 100, where A is the absorbance of the filtrate of the cultures after 10 days of growth and B, the absorbance of control. The factor levels used in both designs are shown in Tables 2 and 3. The data were analyzed using Statistic 6.1 computer program (StatSoft, Tulsa).

RESULTS AND DISCUSSION

The process of decolorization of Orange II and Black V dyes by *T. membranacea* and *P. sanguineus* was investigated in different culture conditions (growth media, shaking speed, luminosity and pH). The obtained results are shown in Table 4.

P. sanguineus showed the highest biomass (7.5 g/l) when grown on King medium supplemented with Black V dye, pH 4.5, without aeration and luminosity. However, when it was grown on King medium with the Orange dye, without agitation, with luminosity and pH 4.5 (flask 12), it

Table 3. Experimental matrix used in the 26-2 fractional factorial design.

Assay	Fungi	Media	Shaker (rev/min)	Lighting regime	pH	Dye
1	*T. membranacea*	Malt	0	dark	4.5	Orange II
2	*P. sanguineus*	Malt	0	dark	5.5	Orange II
3	*T. membranacea*	King	0	dark	5.5	Black V
4	*P. sanguineus*	King	0	dark	4.5	Black V
5	*T. membranacea*	Malt	130	dark	5.5	Black V
6	*P. sanguineus*	Malt	130	dark	4.5	Black V
7	*T. membranacea*	King	130	dark	4.5	Orange II
8	*P. sanguineus*	King	130	dark	5.5	Orange II
9	*T. membranacea*	Malt	0	light	4.5	Black V
10	*P.sanguineus*	Malt	0	light	5.5	Black V
11	*T. membranacea*	King	0	light	5.5	Orange II
12	*P. sanguineus*	King	0	light	4.5	Orange II
13	*T. membranacea*	Malt	130	light	5.5	Orange II
14	*P. sanguineus*	Malt	130	light	4.5	Orange II
15	*T. membranacea*	King	130	light	4.5	Black V
16	*P. sanguineus*	King	130	light	5.5	Black V

Table 4. Efficiency of decolorization of Orange II and Black V dyes and biomass of *T. membranacea* and *P. sanguineus*.

Assay	Fungi	DT1	BT1 (g/L)	DT2	BT2 (g/L)	DT3	BT3 (g/L)
1	T.m	41	2.5	61	5.0	74	5.0
2	P.s	18	2.5	43	5.0	51	5.0
3	T.m	24	2.5	36	7.5	43	7.5
4	P.s	29	2.5	57	2.5	86	7.5
5	T.m	95	2.5	97	5.0	97	7.5
6	P.s	89	2.5	96	2.5	97	5.0
7	T.m	70	5.0	91	5.0	92	7.5
8	P.s	94	2.5	96	5.0	97	5.0
9	T.m	37	2.5	79	5.0	87	7.5
10	P.s	50	2.5	62	5.0	79	5.0
11	T.m	80	2.5	90	7.5	94	7.5
12	P.s	91	2.5	91	2.5	91	2.5
13	T.m	98	5.0	99	7.5	99	7.5
14	P.s	96	2.5	97	5.0	98	5.0
15	T.m	50	5.0	67	5.0	78	7.5
16	P.s	50	2.5	57	5.0	93	7.5

T.m*, T. membranacea*; P.s*, P. sanguineus;* DT1, discoloration at time 1 (6 days); DT2, discoloration at time 2 (12 days); DT3, discoloration at time 3 (18 days); BT1, biomass at time 1; BT2, biomass at time 2; BT3, biomass at time 3.

had the lowest biomass, although it was effective in decolorization the dye. Overall, the biomass production by *T. membranacea* was superior in comparison to the other species studied, with the biomass of 7.5 g/l under the growing conditions tested (except for flask 3).

The two fungal isolates were efficient in decolorization of Orange II in the first week of growth, reaching values ranging from 70 to 98% in the different forms of cultivation, except when they were cultured on malt, without agitation and without light, showing a percentage of decolorization below 42%. The maximum decolorization rate (98%) for the Black V dye was achieved with the *P. sanguineus* when cultured in malt medium, at 130 rpm shaking speed, in the presence of light and pH 4.5.

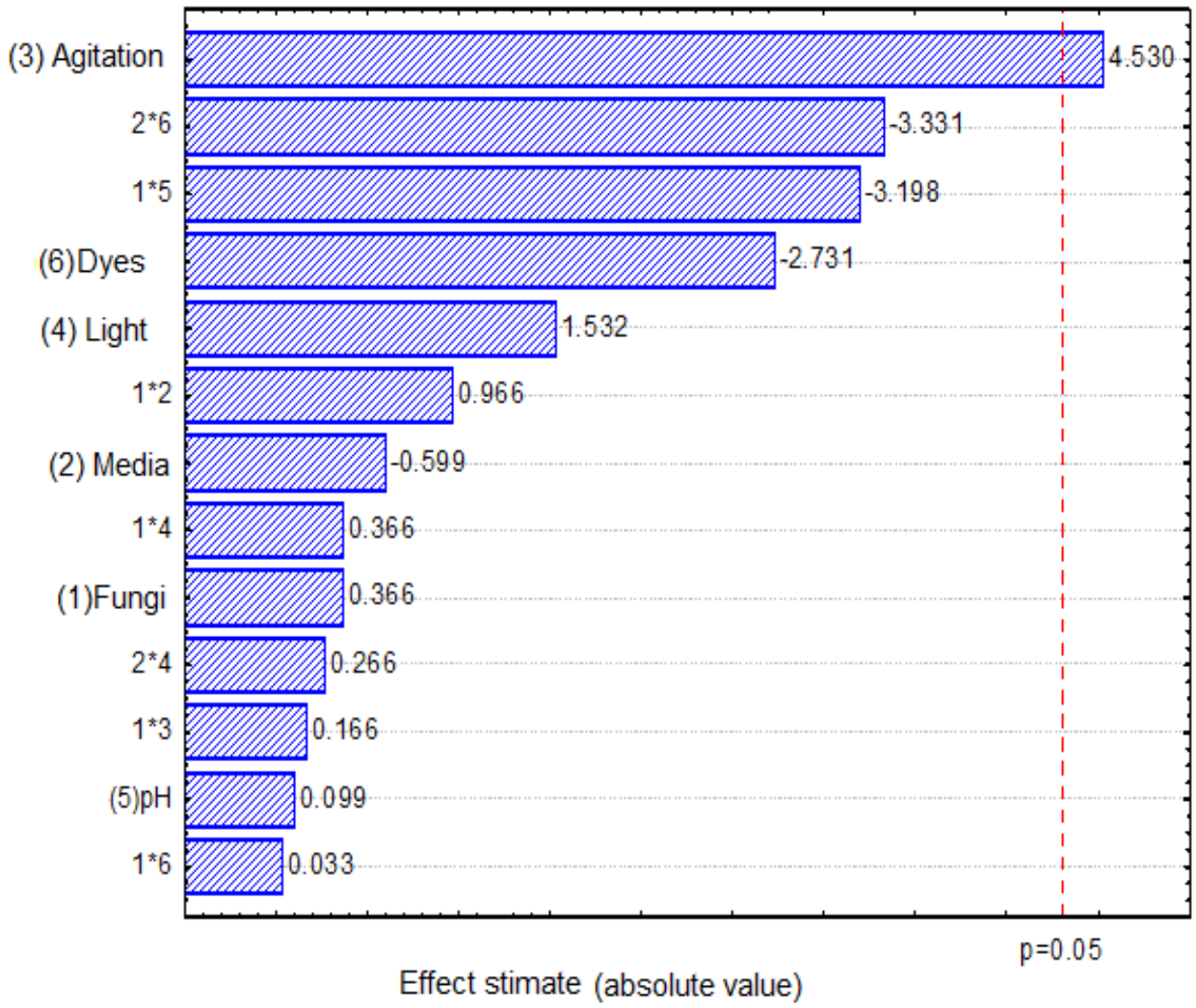

Figure 1. Pareto chart of effects on the response degradation on the 6th day of growth.

Similar results were obtained by Novotný et al. (2004), as they assessed the ability of the fungus *Irpex lacteus*, after two weeks of growth in liquid medium containing the Black V dye, under different growing conditions. Tychanowicz et al. (2004) correlated the ability of the white rot fungus *Pleurotus pulmonarius* to decolorize industrial dyes with its production of phenoloxidases.

The Pareto charts (Figures 1 and 2) represent the estimated effects of the variables studied (main effect or first order) and interactions between variables (second order effect) on the response variable (decolorization percentage of dyes by fungi) in order of magnitude, in the sixth and twelfth days after incubation. The length of each bar is proportional to the standardized effect of the variable. The vertical line can be used to evaluate that the effects were statistically significant, that is, the bars that extended through this line correspond to statistically significant effects with a confidence level of 95%.

The significance (P = 0.05) of the six variables employed in the fractional planning, as well as the interactions between them is shown in Figure 1. The variables and their interactions are represented by symbols and numbers on the vertical axis. The analysis of interactions between the variables showed that agitation was the only significant additional effect on the percentage of decolorization of dyes on the 6th day, regardless of the isolate. Sevil and Buket (2011) also revealed an increase in the decolorization of synthetic dyes when the fungal species were subjected to conditions of agitation. According to Tuor et al. (1995), aeration is one of the environmental conditions that may interfere with enzymatic activity of fungal decomposers of wood, increasing their efficiency in reducing the color of the dye. Researchers have shown that agitation of fungal species in liquid media increases the efficiency of the isolates in the color removal of dyes. Swamy and Ramsay (1999)

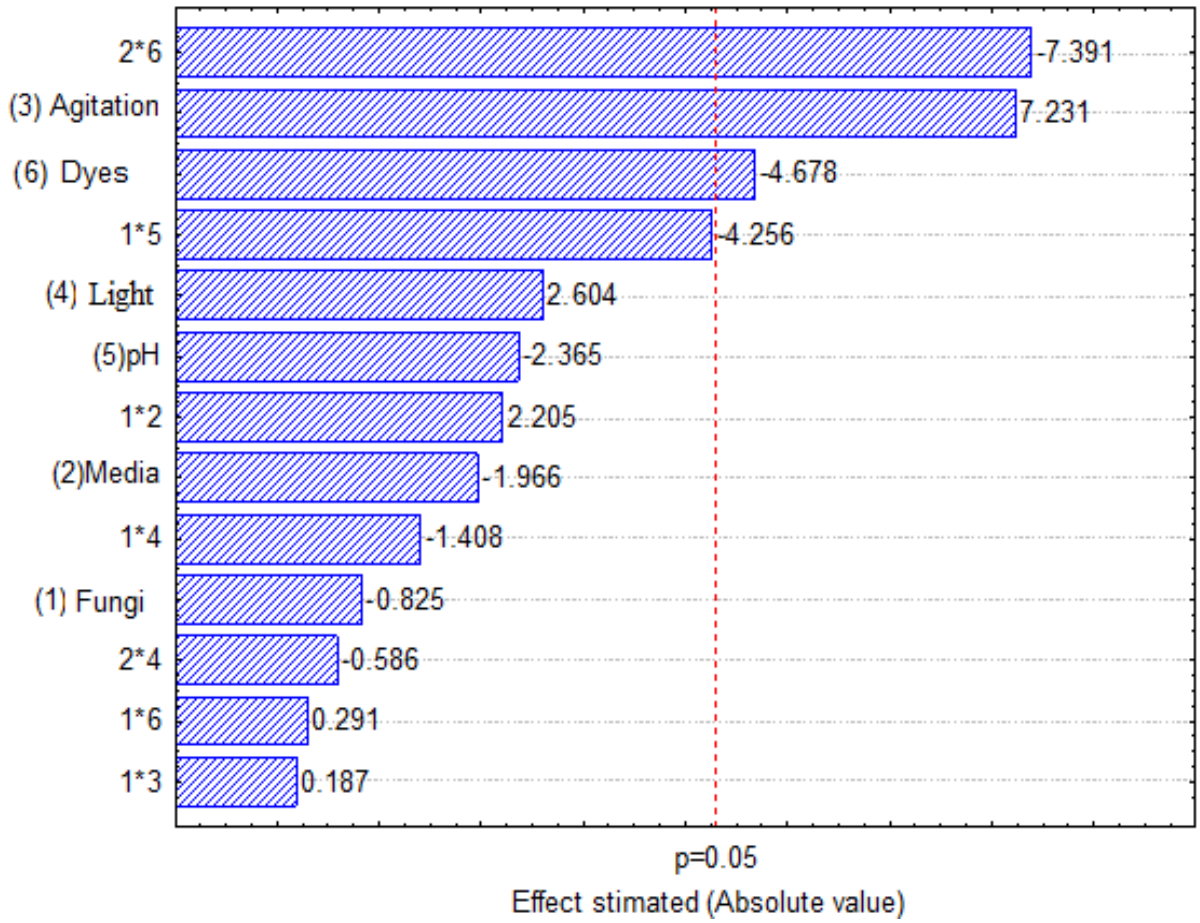

Figure 2. Pareto chart of effects on the response degradation on the 12th day of growth.

observed that the percentage of decolorization of azo dyes was higher with the fungi *Phanerochaete chrsisoporium, Trametes versicolor* and *Bjerkandera* when subjected to agitation. The same was reported by Ge et al. (2004) as they subjected *Phanerochaete sordida* to an increased rotational speed (rpm). Cultural conditions can affect the physiology and metabolism of the fungus, activating its enzymes.

In Figure 2, the variables present agitation and dyes, singly or in combination, and significant additive effects on decolorization of dyes used on the twelfth day of growth. Evaluating the variable agitation, it appears that its effect was positive and it means that the shaking of the flasks at 130 rpm was the best condition for the decolorization of dyes on the 12th day of growth. Regarding the second variable (dyes), it was also significantly affected; moreover, the culture medium interferes with the percentage of decolorization of dyes by the fungi selected. When the two variables are associated (aeration and culture medium), the interactive effect is as large as possible. This kind of effect on the percentage of color removal of synthetic dyes was observed with white-rot fungi by other authors (Sathiya moorthi et al., 2007; Sevil and Buket 2011).

The interaction between variables, culture media and kinds of dyes, had a significant effect. This means that a variable does not act alone on the responses, its effect depends on another variable. This interaction effect is more evident in the graphs of geometric interpretation in Figure 3. It is observed that the Black V dye was better bleached when the fungi grew in malt medium. As for the Orange II that was cultivated in two ways, the fungi showed high degradation capacities of the dye; however, in King medium, the optimization of this process occurred.

The biomasses (dry weight) of fungi studied were evaluated at six, 12 and 18 days. The increase in biomass of fungi is not necessarily reflected in the increase in the percentage of decolorization of the dyes evaluated. On the 18th day of growth, although there was an increased percentage of discoloration, no significant increase for the two responses was seen (percentage of decolorization and biomass). However, Yonne et al., (2004) found a relationship between the fungal biomass and

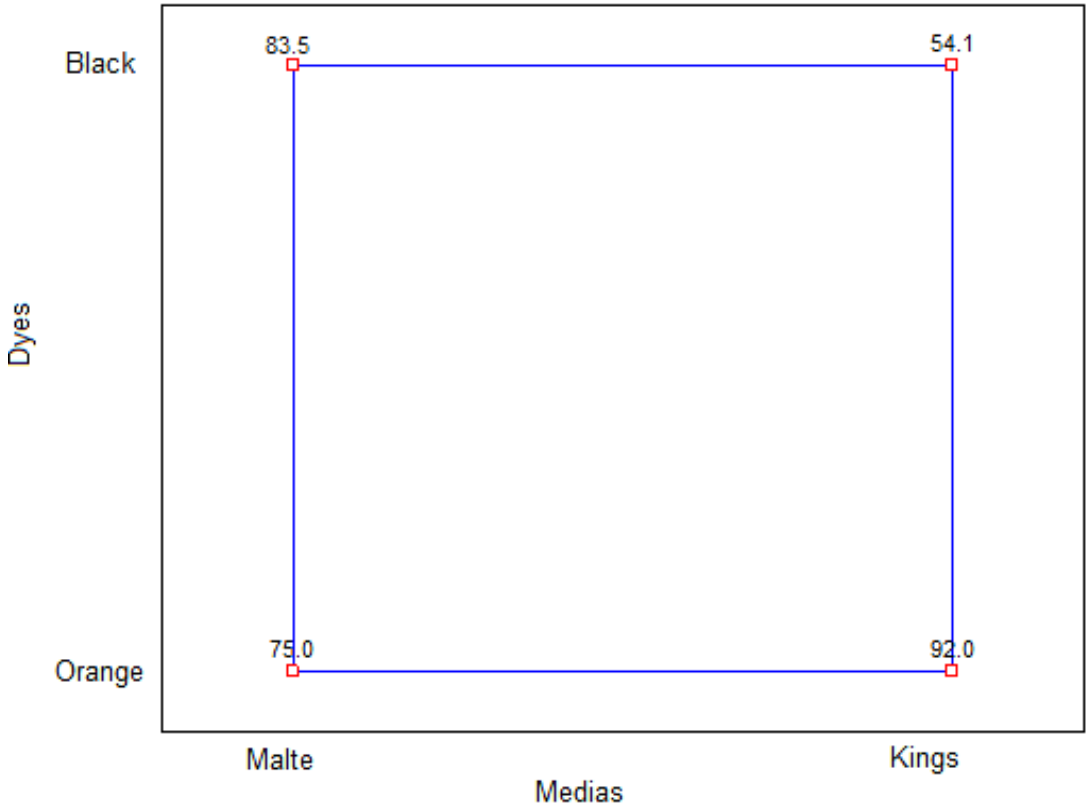

Figure 3. Diagram for geometric interpretation of the effects of planning (2^{6-2}) on the 12th day of growth.

enzymatic activity of manganese peroxidase (MnP), with a directly proportional relationship between enzyme production and the increase in biomass of white rot fungi.

Conclusions

The results show that *T. membranacea* and *P. sanguineus* were efficient in decolorization of the dyes investigated in all culture conditions tested in this study, suggesting their use in the treatment of textile dyes. However, the decolorization rate of Orange II was the maximum in pH 5.5, malt medium, shaking speed of 130 rpm in the presence of light for the fungus *T. membranacea* and pH 4.5, malt medium, shaking speed of 130 rpm in the presence of light for the fungus *P. sanguineus*. The optimum decolorization rate for Black V was at pH 5.5, malt medium, shaking speed of 130 rpm in the dark (*T. Membranacea*) and pH 4.5, malt medium, shaking speed of 130 rpm in the dark (*P. sanguineus*).

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