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Decolorization of methylene blue by new fungus: *Trichaptum biforme* and decolorization of three synthetic dyes by *Trametes hirsuta* and *Trametes gibbosa*

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ABSTRACT

In order to screen dye decolorization by *Trametes hirsuta*, *Trametes gibbosa* and new fungus *Trichaptum biforme*, Methylene Blue (MB), Methyl Green (MG) and Reactive Black 5 (RB5) were studied in Broth culture at concentrations of 25, 50 and 70 ppm. Decolorization of these three kind dyes by *T. hirsuta* decreased by increasing of concentration. Decolorization of RB5 by *T. hirsuta* was examined in two different medium contains of Mn^{2+} or veratryl alcohol (VA), as activators. At the presence of laccase activator (VA), RB5 was decolorized 91% at concentration of 70 ppm, and in the medium contains of MnP activator (Mn^{2+}), dye was decolorized less than 50% at same concentration. Decolorization of RB5 by *T. hirsuta* was more than decolorization of dye at the presence of MnP activator (Mn^{2+}). *T. gibbosa* decolorized MB, MG and RB5 more than 80% in the presence of MnP activator. Decolorization of the three kind dyes by *T. gibbosa*, was independent of concentration. *T. gibbosa* decolorized different dyes during 20 days and *T. hirsuta* did it during 32 days, this is due higher potential of decolorization of synthetic dyes for *T. gibbosa* than *T. hirsuta*. Decolorization of MB by new fungus *T. biforme* was studied in two medium contains of MnP activator and laccase activator. Rate of decolorization in the presence of MnP activator was more than decolorization in the presence of laccase activator. Decolorization of MB by new fungus *T. biforme* was higher than decolorization of MB by two other fungi.

1. Introduction

Environmental pollution containing recalcitrant xenobiotic materials has become one of the major ecological problems. Many of these compounds are major environmental pollutants such as nitrotoluenes, dioxin, organic acid, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pesticides, synthetic dyes, wood preservatives, synthetic polymers and olive mill wastewater [1-5]. Most of these compounds are toxic, carcinogenic and highly resistant to degradation [6-8]. Synthetic dyes are extensively used in a number of industries, such as textile dyeing or paper printing. Synthetic dyes represent a large group of chemically different compounds, which are classified by their chromophore as azo, anthraquinone, triphenylmethane, heterocyclic or phthalocyanin dyes [9,10]. The total world colorant production is estimated to be about 800000 ton/year. More than 10000 dyes are commercially available and at least 10% of the used dyestuff enters the environment through wastes [8,11-16]. The elimination of such dye-containing effluents is mostly based on physical and chemical procedures, e.g. adsorption, concentration, chemical transformation and incineration. These methods are rather costly and sometimes produce hazardous products [3,10,17-19]. Therefore, several investigations have been carried out several investigations to identify a cost effective and environmentally acceptable technology that could be applied to remediate the contamination. Bioremediation is one of these techniques that involve almost exclusively microbial processes [20-23]. White rot fungi have been studied as one of the possible agents of biodegradation since their extracellular degradation systems are basically non-specific. This fact that allows the degradation of mixture refractory substances and can be applied to environmental pollutants [24,25].

The primary source of carbon for the white rot fungi is trees and plant cellulose, which is protected by a complex polymer known as lignin. The lignin degrading enzymes such as Manganese peroxidase (MnP) (EC 1.11.1.13), Lignin peroxidase (LiP) (EC 1.11.1.14) and Laccase (Lac) (EC 1.10.3.2) degrade lignin [26,27]. Taxonomically, white rot fungi are mostly basidiomycetes, and a few ascomycetes are also capable of white rot decay [28]. Fungal strains have different ligninolytic systems which distinguish them from each other [16,23,29-30]. Laccase requires oxygen and peroxidase need oxygen peroxide for reactions. *Phanerochaete chrysosporium* was the first fungus that was examined its ability to decolorization [31,32]. A fungus species can degrade pollutants. In contrast of fungi a consortium of bacteria may be needed to completely degrade the same mixture. Also bacteria which rely on various enzymes must first adsorbe the chemicals. Then these chemicals induce the production of enzymes needed for degradation [23]. Hitherto has been studied on ability of different fungi species in dye decolorization [28]. Kling and Neto (1991) [33], studied oxidation of MB by crude lignin peroxidase from *Phanerochaete*

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chrysosporium. This reaction depended on peroxide concentration. Fungus *Coriolos versicolor* decolorized Methylene Blue at concentrations 5 and 10 mg/L. At this study various glucose and $NH_4H_2OPO_4$ were effective in rate of decolorization [34].

2. Experimental

2.1. Effect of temperature on growth of fungi

Trametes hirsuta (Fr.) Pilát., *Trametes gibbosa* (Pers.) Fr. and *Trichaptum biforme* (Fr.) Ryvarden were collected in April 2008 from Abbas Abad Forest, in North of Iran. The fungi were growing on dead wood of hornbeam (*Carpinus betulus* L.) from *Betulaceae.* Specimens were identified based on morphological features according to Ryvarden and Gilbertson [35]. *T. hirsuta* based on following features was identified: Basidiocarp annual, effused-reflexed, upper surface hirsute, gray, with brownish margin, cap 8 cm wide, 5 cm long and 2 cm thick. Context trimitic with clamp connection. Hyphal system trimitic. Pores white, 3 per mm. Spores 5*2 µm, smooth, cylindric. Inedible. *T. gibbosa* according to these features was identified: Basidiocarp annual, applanate, semicircular, grayish white, upper surface colored green due to algal growth. Pores creamy-white, elongated and stollike, 2 per mm. Hyphal system trimitic. Spores hyaline, clyndric, 4.5*2.5 µm. *T. biforme* according to following characters was identified: Basidiocarp annual, imbricate, dimidiate, hirsute to glabrous with age, surface violaceous. Pore surface purple, the pores angular, 5 per mm, tube layer violaceous. Hyphal system dimitic. Cyctidia abundant. Basidiospores cylindric, hyaline, smooth, 6*2.5 µm. To obtain pure culture without contamination, small fragments about 1 mm^3 from the inner flesh of the basidiocarps of *T. hirsuta*, *T. gibbosa* and *T. biforme* were plated into petri dishes contains of medium culture (Malt Extract Agar (M.E.A.) is the best medium culture for Basidiomycetes) separately and mycelium growing, were repeatedly transferred into new plates until the culture were pure. To ensure that the mycelium growing are *T. hirsuta*, *T. gibbosa* and *T. biforme*, mycelium were observed with a optical microscope. Existance clamp connection, confirmed the desired fungal growth. Stock cultures were stored in M.E.A. plates at 4 °C and periodically subcultured.

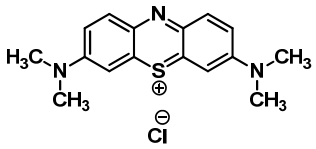
Not all white rot fungi are able to produce all enzymes including laccase, Mn peroxidase (MnP) and Lignin peroxidase (LiP). Different 5 mm Agar plugs from the growing margin of *T. hirsuta, T. gibbosa* and *T. biforme* were inoculated into malt agar plates. Replicates were prepared for fungus and incubated at each of the following temperatures (15, 20, 25, 30, 35, 40, 50 and 60 °C). The experiment was repeated three times. Growth was followed by measuring radial extension of the mycelium (every 24 h) for at least 15 days. Mean growth rates (mm/day) were calculated, to find the optimum growth temperature.

2.2. Study of optimum pH for growth of mycelium

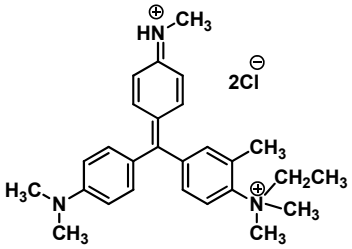
To study the effect of pH on growing fungi, mycelia were incubated on Malt Extract Broth at different pH = 4, 6, 7 and 9 during 15 days and dry weight of mycelium were measured every 3 days.

2.3. Dye decolororization experiments

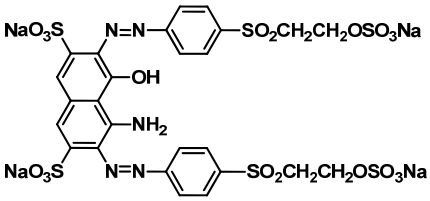
In order to study of decolorization of three structurally different dyes (Scheme 1) MB (Heterocyclic dye), MG (Triphenylmethane dye) and RB5 (Diazo dye), by *T. hirsuta*, *T. gibbosa* and MB by *T. biforme* were selected. These fungi were grown on broth medium. At first two different medium containing MnP activator including Mn^{2+}, according to Mohorčič *et al.* [37] procedure and laccase activator including veratryl alcohol, according to Minussi *et al.* [36] procedure were used.



Methylene Blue; Heterocyclic dye
λ_{max} = 633 nm



Methyl Green; Triphenylmathane dye
λ_{max} = 635 nm



Reactive Black 5; Diazo dye
λ_{max} = 603 nm

Scheme 1

The composition of the culture medium to induce laccase production and activation was prepared according to Minussi *et al.* procedure [36]. The basal medium containing per litre (g/L): malt extract (5.0); peptone (10.0); glucose (20.0); and $CuSO_4.5H_2O$ (0.005), (0.5) mM pyrogallol; veratryl alcohol (laccae activator) (0.07); pH was 4. The medium for screening Mn Peroxidase production by fungus, prepared according to Mohorčič *et al.* procedure [37] per litre (g/L) contain of: Glucose (10.0); Yeast Extract (0.2); tartaric acid (3.0); Tween 80 (1.0); KH_2PO_4 (0.2); $CaCl_2.\ 2H_2O$ (0.146); $(NH_4)_2HPO_4$ (0.157); $MgSO_4.\ 7H_2O$ (50.0 mg); $ZnSO_4.\ 7H_2O$ (42.5 mg); $MnSO_4.\ H_2O$ (MnP activator) (33.8 mg); $CoCl_2.\ 6H_2O$ (7.0 mg); $CuCl_2.\ 2H_2O$ (0.7mg); $FeCl_3$ (0.54 mg); NaCl (0.9 mg). pH was 4.5. All chemicals were obtained from Sigma.

The following synthetic dyes were obtained from Sigma: Methylene Blue (MB), Methyl Green (MG). Reactive Black 5 (RB5) was obtained from a national dyeing factory.

To test the ability of fungi to decolorize synthetic dyes in vivo, each dye was membrane-filtered with a 0.25 µm cellulose nitrate filter and was concentrated to final concentrations of 25, 50 and 70 ppm. The dye disappearance was determined spectrophotometrically (Agilent 8453 UK). Line equation of tested dyes was obtained by Sigma Plot 8.0. Afterward amount of dye absorptions were placed in equation and were obtained dye concentrations. To obtain percentage of dye removal was used Removal Efficiency formula:

$$RE = \frac{\text{Dye concentation in control medium - Dye concentation in medium contain of mycelium}}{\text{Dye concentration in control medium}} \times 100 \quad (1)$$

Table 1.Dry weight of mycelium (g) from three fungal species at different pH.

pH	Day 3		Day 6		Day 9		Day 12		Day 15	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
T. hirsuta										
4	0.6521	0.0023	0.9626	0.1023	1.0678	0.0833	1.4984	0.1787	1.6251	0.0921
6	0.5110	0.0982	0.6878	0.0832	0.9991	0.6231	1.0023	0.0928	1.1233	0.1972
7	0.2930	0.2342	0.3940	0.0099	0.3999	0.1798	1.0065	1.0382	1.0382	0.3232
9	0.0283	0.7002	0.0232	0.3987	0.2331	0.4862	0.3921	0.6523	0.5332	0.0909
T. gibbosa										
4	0.9210	0.0078	1.2039	0.7577	1.5321	0.1827	1.6342	0.1872	1.6661	0.0123
6	0.5726	0.3983	0.5999	0.2378	0.6876	0.4726	0.7917	1.0480	0.9899	0.8277
7	0.5111	0.0887	0.5290	0.0972	0.5654	0.0782	0.5920	0.1873	0.6287	0.0343
9	0.2167	0.2768	0.3432	0.1762	0.3249	0.0637	0.3498	0.1077	0.3578	0.9733
T. biforme										
4	0.0977	0.0763	0.4877	0.5165	0.6890	0.0761	0.7463	0.0370	0.7300	0.0721
6	0.2890	0.5621	0.4170	0.0125	0.6100	0.0127	0.6909	0.0232	0.7023	1.0990
7	0.2973	0.0827	0.2999	0.1878	0.3100	0.0232	0.6763	0.0883	0.7011	0.9820
9	0.0230	0.6162	0.0993	0.1657	0.2983	0.0072	0.3012	0.9792	0.4977	0.0400

3. Results and discussion

3.1. Effect of temperature on growth of fungi

Optimum temperature for *T. hirsuta*, *T. gibbosa* and *T. biforme* was 35, 30 and 25 °C, respectively. Optimum temperature for pured laccase and MnP activity was 40 to 60 °C [28], but at this experiment decolorization was determined in vivo and at these temperatures growing of both *T. hirsuta*, *T. gibbosa* and *T. biforme* stoped. Therefore the experiments were followed at 35, 30 and 25 °C, respectively.

3.2. Study of optimum pH for growth of mycelium

Three fungal mycelium at different studied pH = 4, 6, 7, and 9 were compared at Table 1. Dry weight of mycelium (g) from three fungal species at different pH and times were measured. Therefore optimum pH for highest growth of *T. hirsuta*, *T. gibbosa* and *T. biforme* was 4 (Table 1).

3.3. Dye decolorization experiments

3.3.1. Decolorization by Trametes hirsuta

Three dyes (MB, MG and RB5) were examined at three concentrations of 25, 50 and 70 ppm in medium culture containing the Mn^{2+} (Mn peroxidase activator). MB at concentrations of 25, 50 and 70 ppm was degraded more than 80% at 16th day. It is agree to Novotný *et al.* [3] that reported *Irpex lacteus* (Fr.) Fr. Decolorized MB by 80% within two weeks. This experiment was continued until 32 day. Rate of decolorization was reduced by increasing concentration, so that at concentration of 25 ppm, MB was degraded completely (100%) and at 50 and 70 ppm, MB degraded 90% and 80% respectively (Figure 1).

Rate of decolorization of MG at concentration 25 ppm was less than decolorization at concentrations 50 and 70 ppm until 22th day, but in following days, rate of decolorization was achieved more than 50 and 70 ppm. Dye was decolorized completely at 24th day. MG was decolorized at concentrations 50 and 70 ppm at 32th day (Figure 2). Results showed decolorizatiion of MB was more than MG by *T. hirsuta*, it is agree with this report that highly substituted triphenylmethane dye such as MG required longer time to be decolorized or could only be decolorized to a certain extent [38].

RB5 was decolorized 91% at concentration of 70 ppm in medium contains of laccase activator (VA), but in the medium contains of MnP activator (Mn^{2+}), dye was decolorized less than 50%. This dye was decolorized completely at concentrations 25 ppm and 50 ppm in the presece of VA while in the presece of Mn^{2+} rate of decolorization was achieved 65% (Figure 3 and 4). RB5 was decolorized slower than MB and MG, because of its complex structure. It is notable that some compounds that were rapidly decolorized by MnP were resistant to other enzymes and the other compounds which are resistant to MnP [39]. Moreover, λ_{max} of RB5 was changed during study. λ_{max} of RB5 changed from 602 at day one to 610 at day 8 and to 616 and 620 at days 16 and 32, respectively. Therefore RB5 may be converted to other dyes products and this delay the decolorization. Spectrum of MB and MG showed complete decolorization.

Rodríguez Couto *et al.*, [40] studied of the effect of redox mediators (2,20-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1-hydroxybenzotriazole (HBT) Remazol Brilliant Blue R (RBBR) on synthetic dyes: Sella Solid Red and Luganil Green by laccase from *Trametes hirsuta.* Result showed higher activities of enzyme in the presence of mediators than those obtained without mediators addition. HBT showing a decolourization percentage of 88% in 10 min for Sella Solid Red and of 49% in 20 min for Luganil Green.

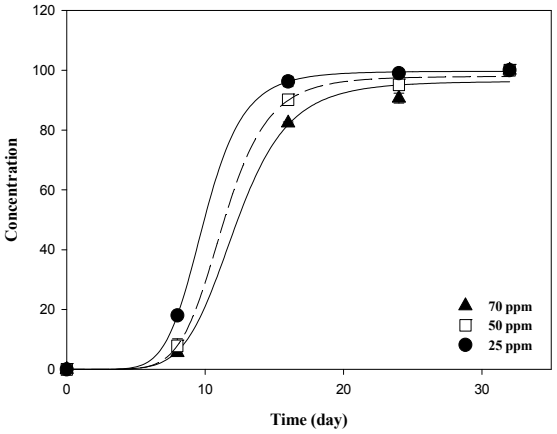


Figure 1. Removal Efficiency (%) of MB at three different concentrations within 32 days by *T. hirsute.*

3.3.2. Decolorization by T. gibbosa

Decolorization of MB, MG and RB5 by *T. gibbosa* was studied during 20 days at three concentrations (25, 50 and 70 ppm) in medium contains of Mn^{2+}. MB was degraded completely at concentration of 25 ppm at 20th day. Decolorization of MG, MB and RB5 was independent of concentration. Decolorization rate of MB at 70 ppm, was less than 50 ppm in the first 8 days, but in following days the rate of decolorization at 70 ppm was more than 50 ppm (Figure 5).

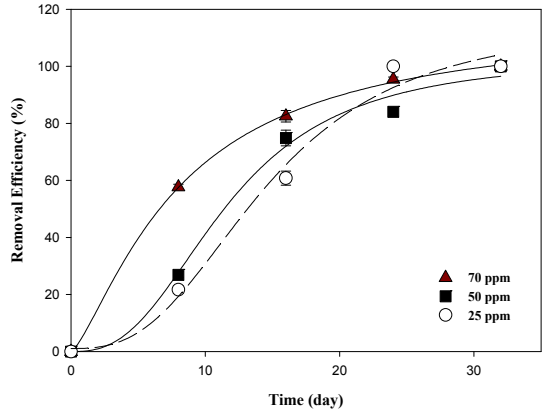


Figure 2. Removal Efficiency (%) of MG at 25, 50 and 70 ppm by *T. hirsuta* in medium contain of MnP activator within 40 days.

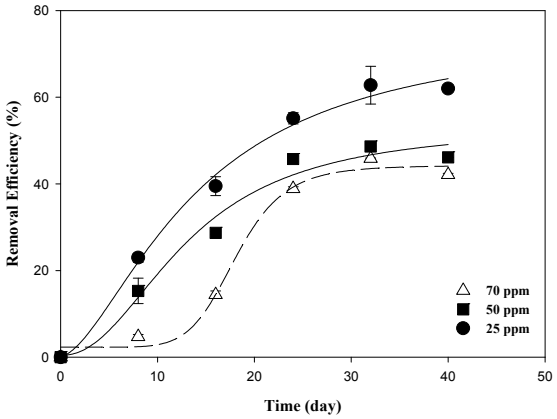


Figure 3. Removal Efficiency (%) of RB5 in medium contain of MnP activator within 40 days by *T. hirsute*.

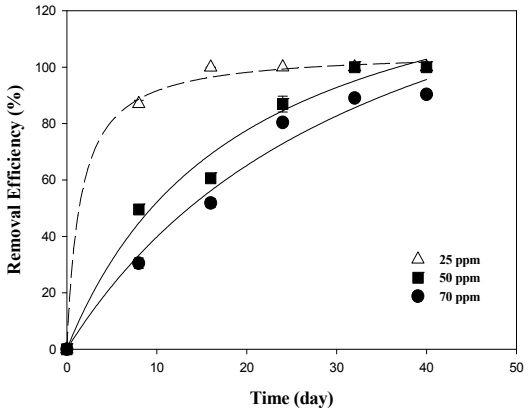


Figure 4. Removal Efficiency (%) of RB5 in culture contain of Laccase activator within 40 days *by T. hirsute*.

MG was decolorized completely (100%) at concentration of 25 at 8th day. At concentrations 50 and 70 ppm, MG was decolorized 97% and 80% at 20th day (Figure 6).

RB5 was decolorized completely (100%) at 16th day. Dye was decolorized more than 80% at 20th day at 50 and 70 ppm, (Figure 7). Figures showed decolorization of MG was more than MB by *T. gibbosa*, that it is not agreed with Osma *et al*. that expressed highly substituted Triphenylmathane need more time to decolorization [38]. It is likely that in addition to dye structure is effective in the decolorization, the type of fungus is effective in the decolorization too [38]. The decolorization of Reactive Black 5 (RB5) by immobilised *Funalia trogii* was investigated by Mazmanci and Unyayar [41]. The effect of mycelial age was studied, and decolorization rate of a 3-day-old age culture was higher than those of 0- and 6-day-old cultures. The growth of *F. trogii* was inhibited by all tested dye concentrations with compared to controls.

Reactive Black 5 was used in screening 25 fungal strains. It was shown that the concentrations of the constituents had to be reduced to allow fungal growth. Purified manganese peroxidase prepared from *Bjerkandera adusta* was tested for decolorization of several artificial dye baths. Based on absorbance units, the largest reduction was achieved with the Reactive Black 5 and Acid Orange 7 dye baths [37].

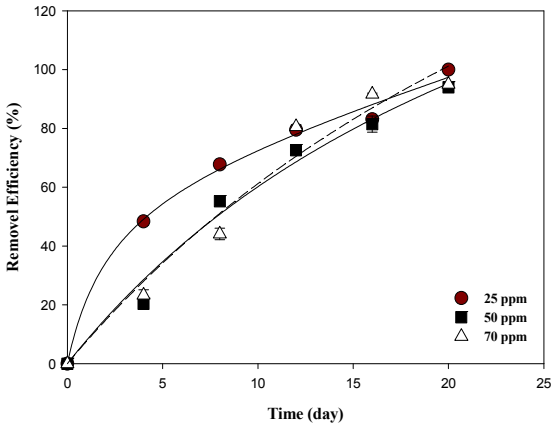


Figure 5. Removal Efficiency (%) of MB by *T. gibbosa* within 20 days.

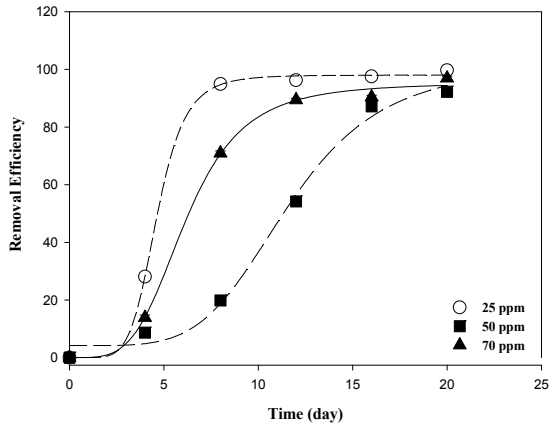


Figure 6. Removal Efficiency (%) of MG by *T. gibbosa* during 20 days.

3.3.3. Decolorization by Trichaptum biforme

Decolorization of MB was studied in two medium contains of MnP activator and laccase activator by new fungus *T. biforme* during 20 days. Maximum decolorization of MB in medium culture contains of Mn^{2+} was achieved 40% and in medium contains of VA was achieved 98% at 20th day (Figure 8 and 9). According to cures, can be claimed MnP is the main enzyme of *T. boforme* in dye decolorization. The most important result obtained is that in medium contain of MnP activator, new fungus *T. biforme*, decolorized MB higher than two other species.

Table 2. Dry weight of mycelium (g) from three fungal species at different concentrations (ppm) of three kind dye.

	Day 0		Day 8		Day 16		Day 24		Day 32	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
T. hirsuta at present of MB										
25 ppm	0	0	0.0759	1.0001	0.3472	0.4630	0.3515	0.6032	0.4001	0.0984
50 ppm	0	0	0.1020	0.1009	0.3116	0.2630	0.2480	0.1231	0.3045	0.1211
70 ppm	0	0	0.0826	0.0039	0.3116	0.0023	0.2839	0.1290	0.3133	0.0824
Control	0	0	0.1972	0.0763	0.3016	0.0342	0.4888	0.0553	0.6380	0.6101
T. hirsuta at present of MG										
25 ppm	0	0	0.1275	0.0923	0.2686	0.4350	0.2135	0.3092	0.2240	0.0621
50 ppm	0	0	0.1456	0.4362	0.2855	0.0211	0.2382	0.1097	0.2400	0.0626
70 ppm	0	0	0.1578	0.0753	0.3202	0.3430	0.2696	0.5372	0.2690	0.0200
Control	0	0	0.2258	0.3525	0.3362	0.2542	0.4687	0.9999	0.6599	0.0201

	Day 0		Day 8		Day 16		Day 24		Day 32		Day 40	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
T. hirsuta at present of RB5 and MnP activator												
25 ppm	0	0	0.076	0.0380	0.0850	1.0972	0.1150	0.0821	0.1320	0.1452	0.1500	0.2234
50 ppm	0	0	0.0450	0.0983	0.0735	0.0762	0.0915	0.5442	0.1112	0.3410	0.1187	0.1650
70 ppm	0	0	0.0355	0.0732	0.0700	0.0722	0.0895	0.1600	0.1010	0.4726	0.1000	0.2076
Control	0	0	0.0271	0.0029	0.0410	0.0650	0.0574	0.0032	0.1021	0.2280	0.1106	0.1092
T. hirsuta at present of RB5 and laccase activator												
25 ppm	0	0	0.0649	0.0901	0.1886	0.0452	0.1924	0.2870	0.2163	0.0541	0.2320	0.5200
50 ppm	0	0	0.1248	0.1652	0.2498	0.0521	0.2607	0.0175	0.2845	0.4007	0.2913	0.3120
70 ppm	0	0	0.1408	0.2571	0.2361	0.0099	0.2985	0.2208	0.3362	0.1940	0.3872	0.1209
Control	0	0	0.2258	0.0123	0.3016	0.2310	0.4687	0.7610	0.6599	0.6520	0.7010	0.0212

	Day 0		Day 4		Day 8		Day 12		Day 16		Day 20	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
T. gibbosa at present of MB												
25 ppm	0	0	0.0730	0.0342	0.1721	0.0736	0.3328	0.9660	0.3210	0.0572	0.3019	0.2222
50 ppm	0	0	0.0841	0.0432	0.1345	0.2611	0.3401	1.2323	0.3105	0.2078	0.2854	0.0632
70 ppm	0	0	0.1011	0.6540	0.1899	0.1203	0.3542	0.3230	0.3540	0.2532	0.2894	0.3034
Control	0	0	0.1121	0.5420	0.2500	0.3232	0.2891	0.1235	0.3120	0.0162	0.3355	0.0834
T. gibbosa at present of MG												
25 ppm	0	0	0.1367	0.3650	0.1621	0.0343	02698	0.0213	0.3500	0.0322	0.3121	0.0632
50 ppm	0	0	0.1811	0.6300	0.2710	0.1763	0.3287	0.0232	0.3801	0.8830	0.3289	0.5432
70 ppm	0	0	0.1865	0.0937	0.2141	0.2310	0.2756	0.3420	0.3435	0.3200	0.2178	0.0521
Control	0	0	0.1298	0.2432	0.2509	0.2123	0.2911	0.0323	0.3111	0.3280	0.3120	0.0231
T. gibbosa at present of RB5												
25 ppm	0	0	0.0689	0.2222	0.1432	0.0239	0.1784	0.5332	0.2461	1.0320		
50 ppm	0	0	0.0721	0.2093	0.1626	0.9343	0.1411	0.0434	0.2698	0.2373		
70 ppm	0	0	0.0688	0.0099	0.0854	0.3043	0.1532	0.3432	0.1987	0.2123		
Control	0	0	0.1189	0.1023	0.2487	0.0342	0.2811	0.0994	0.3210	0.1235		
T. *biforme* at present of MB												
25 ppm	0	0	0.0800	0.0099	0.1045	0.4623	0.1220	0.2673	0.1324	0.7222	0.1220	0.2362
50 ppm	0	0	0.0361	0.2782	0.0511	0.1977	0.0575	0.7522	0.0656	0.0723	0.1022	0.2366

3.4. Effect of dye on dry weight of mycelium

To obtain the amount of fungal growth in the presence of dye, dry weight of mycelium which was grown at different concentrations of dyes, was determined (Table 2). Dry weight of mycelium of *T. hirsuta* in medium culture contains of MB was more than MG and RB5. These results showed less toxicity of MB in comparison with other dyes. Also different concentrations of MB did not show any significant effect on fungal growth. In medium containing the MG, mycelium growth decreased with increasing concentration. In medium containing RB5 and laccase activator, dry weight increased surprisingly with increasing dye concentration. In MnP medium in the presence of RB5, maximum growth was achived in 25 ppm. Dye at 50 and 70 ppm was toxic and reduced the mycelium growth, therefore, in medium contains of MnP activator and RB5, either growth of mycelium and decolorization decreased by increasing dye concentration. In laccase activator medium, fungal mycelium could obtain essential materials from culture for growing, therefore produce enzyme and degrade dye. Also was compared dry weight of *T. hirsuta* in medium containing of three dyes at 25 ppm. These results confirm finding about less toxicity of MB, and preference of laccase activator medium in the decolorization of RB5.

Growth of *T. gibbosa* at different concentrations of MB, MG and RB5 in comparison with control medium was achived. Results showed MB and MG are less toxic for *T. gobbosa.* In medium containing the RB5, mycelium growth significantly decreased. These results correlate with data of (Figure 5-7).

Dry weight of *T. biforme* at concentration of 25 ppm was obtained more than 50 ppm (Table 2).

4. Conclusion

We studied dye decolorization of three different synthetic dyes (Remazol Black 5, Diazo dye; Methylene Blue, Heterocyclic dye; Methyl Green, Triphenylmethane dye) by *Trametes hirsuta*, *Trametes gibbosa* and *Trichaptum biforme* from white rot fungi. At this study was determined kind of fungus is effective on decolorization as *T. gibbosa* decolorized MG (has highly substituted and need more time to decolorization) faster than MB while *T. hirsuta* could not do it. Decolorization of MB by new fungus *T. biforme* in medium contains of Mn^{2+} was more than decolorization of this dye in medium contains of VA that showed MnP is main enzyme of *T. biforme* in dye decolorization. The most important result is new fungus *T. biforme* decolorized MB higher than two other species and can more testing be done on it and could be used in future.

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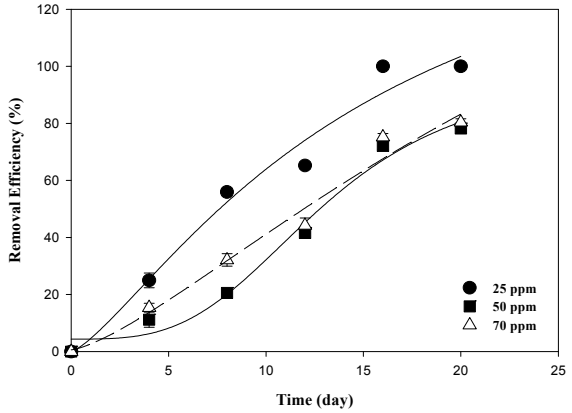

Figure 7. Removal Efficiency (%) of RB5 by *T. gibbosa* within 20 days.

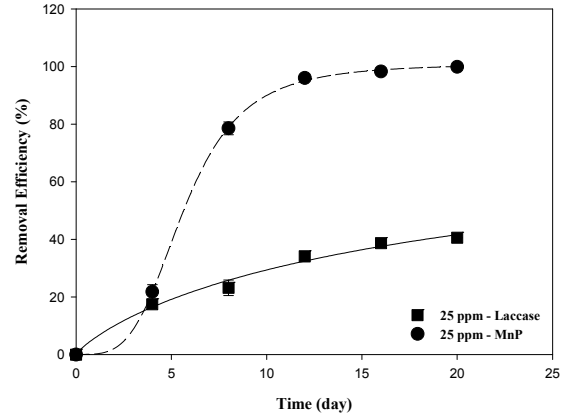

Figure 8. Comparison of removal efficiency of MB by *T. biforme* at concentration of 25 ppm in medium contain of laccase activator and MnP activator.

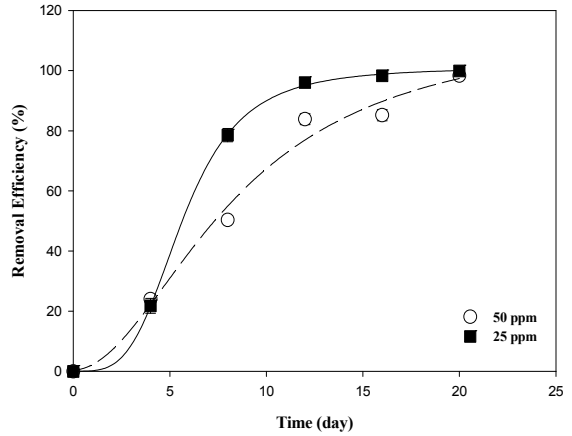

Figure 9. Removal efficiency of MB by *T. biforme* at concentrations of 25 ppm and 50 ppm, in medium contain of MnP activator.

References

[1]. Bayman, P.; Radkar, G. *Int. Biodeterior. Biodegrad.* **1997**, *39*, 45-53.
[2]. Hammel, K. E.; Moen, M. A. *Enzyme Microb. Technol.* **1991**, *13*, 15-18.
[3]. Novotny, C.; Svobodova, K.; Kasinath, A.; Erbanova, P. *Int. Biodeterior. Biodegrad.* **2004**, *54*, 215-223.
[4]. Ollikka, P.; Alhonmaki, K.; Leppanen, V. M.; Glumoff, T.; Raijola, T.; Suominen, I. *Appl. Environ. Microbiol.* **1993**, *59*, 4010- 4016.
[5]. Pointing, S. *Appl. Microbiol. Biotechnol.* **2001**, *57*, 20-33.
[6]. Eichlerova, I.; Homolka, L.; Nerud, F. *Dyes Pigm.* **2007**, *75*, 38-44.
[7]. Hamedaani, H. R.; Sakurai, A.; Sakakibara, M. *Dyes Pigm.* **2007**, *72*, 157-162.
[8]. Trovaslet, M.; Enaud, E.; Guiavarch, Y.; Corbisir, A. M.; Vanhulle, S. *Enzyme Microb. Technol.* **2007**, *41*, 368-376.
[9]. Eichlerova, I.; Homolka, L.; Benada, O.; Kofronova, O.; Hubalek, T.; Nerud, N. *Chemosphere*. **2007**, *69*, 795-802.
[10]. Robinson, T.; McMullan, G.; Marchant, R.; Nigam, P. *Bioresour. Technol.* **2001**, *77*, 247-255.
[11]. Abadulla, E.; Tzanov, T.; Costa, S.; Robra, K .H.; Cavaco-Paulo, A.; Gubitz, G. M. *Appl. Environ. Microbiol.* **2000**, *66*, 3357- 3362.
[12]. Asgher, M.; Azim, N.; Bhatti, H. N. *Biochem. Eng. J.* **2009**, *47*, 61-65.
[13]. Levin, L.; Forchiassin, F.; Ramos, A. M. *Mycologia*. **2002**, *94*, 377-383.
[14]. Madhavi, S.; Revankar, S.; Lele, S. *Bioresour. Technol.* **2007**, *98*, 775-780.
[15]. Palmieri, G.; Giardina, P.; Bianco, C.; Fontanella, B.; Sannita, G. *Appl. Environ. Microbiol.* **2000**, *66*, 920-924.
[16]. Palmieri, G.; Cennamo, G.; Sannia, G. *Enzyme Microb. Technol.* **2005**, *36*, 17-24.
[17]. Dominguez, A.; Rodriguez Couto, S.; Sanroman, A. *World J. Microbiol. Biotechnol.* **2005**, *21*, 405-409.
[18]. Lin, S.; Peng, F. *Water Resour*. **1994**, *28*, 277-282.
[19]. Murugesan, K.; Nam, I. H.; Kim, Y. M.; Chang, Y. S. *Enzyme Microb. Technol.* **2006**, *40*, 1662-1672.
[20]. Balba, M. T.; Al-Daher, R.; Al-Awadhi, N. *Environ. Int.* **1998**, *24*, 163-173.
[21]. Kauffmann, C.; Petersen, B. R.; Bjerrum, M. J. *J. Biorheol.* **1999**, *73*, 71-74.
[22]. Walter, M.; Boul, L.; Chong, R.; Ford, C. *J. Environ. Manage.* **2004**, *71*, 361-369.
[23]. Yateem, A.; Balba, M. T.; Al-Awadhi, N. *Environ. Int.* **1998**, *24*, 181-187.
[24]. Boer, C. G.; Obici, L.; de Souza, C. G.; Peralta, R. M. *Bioresour. Technol.* **2004**, *94*, 107-112.
[25]. Quaratino, D.; Federici, F.; Petruccioli, M.; Fenice, M.; D'Annibale, A. *Anton Leeuw Int. J. G.* **2007**, *91*, 57-69.
[26]. Mayer, A. M.; Staples, R. C. *Phytochemistry* **2002**, *60*, 551-565.
[27]. Meysami, P.; Baheri, H. *Adv. Environ. Res.* **2003**, *7*, 881-887.
[28]. Asgher, M.; Bhatti, H. N.; Ashraf, M.; Legge, R. *Biodegradation*. **2008**, *19*, 771-783.
[29]. Keum, Y. S.; Li, Q. X. *Chemospher*. **2004**, *56*, 23-30.
[30]. Glenn, J. K.; Gold, M. H. *Arch. Biochem. Biophys*. **1985**, *242*, 329-341.
[31]. Singh, H. Mycoremediation: fungal bioremediation (Hoboken/New Jersey: John Wiley & Sons, Inc) 2006.
[32]. Zouari-Mechichi, H.; Mechichi, T.; Dhouib, A.; Sayadi, S.; Martinez, A. T.; Martinez, M. J. *Enzyme Microb. Technol.* **2006**, *39*, 141-148.
[33]. Kling, S. H.; Neto, J. S. A. *J. Biotechnol*. **1991**, *21*, 295-300.
[34]. Mazmanci, M. A.; Unyayar, A.; Ekiz, H. I. **2002**, *11*, 254-258.
[35]. Ryvarden. L.; Gilbertson. R. L. European Polypores, Lubrecht & Cramer Ltd., Oslo, 1993.
[36]. Minussi, R. C.; Pastore, G. M.; Duran, N. *Bioresour. Technol.* **2007**, *98*, 158-164.
[37]. Mohorcic, M.; Teodorovic, S.; Golob, V.; Friedrich, J. *Chemospher*. **2006**, *63*, 1709-1711.
[38]. Osma, J. F.; Toca Herrera, J. L.; Rodriguez Couto, S. *Dyes Pigm*. **2007**, *75*, 32-37.
[39]. Gadd, G. M. Fungi in bioremediation (*Cambridge University Press*, New York) 2001.
[40]. Rodriguez Couto, S.; Sanroman, M.; Gubitz, G. M. *Chemosphere* **2005**, *58*, 417-422.
[41]. Mazmanci, M. A.; Unyayar, A. *Process Biochem*. **2005**, *40*, 337-342.